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Research Article
Oral Acute Toxicity and Estrogenic-Like Effects of the Aqueous Extract of *Anthocleista schweinfurthii* Gilg (*Loganiaceae*)

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Abstract

Background and Objective: *Anthocleista schweinfurthii* is used traditionally to manage female infertility and menopausal complaints. This study aimed to evaluate oral acute toxicity and potential estrogenicity of aqueous extract of *Anthocleista schweinfurthii* in ovariectomized rats. **Materials and Methods:** The acute toxicity was evaluated by administration of the extract at unique dose of 2000 mg kg^{-1}. For estrogenic-like activity, thirty animals were sham-operated or ovariectomized. After 84 days of surgery, six groups of five rats each were daily treated orally during 28 days with: Distilled water for group 1 (sham-operated) and group 2 (ovariectomized), estradiol valerate (group 3) and the 3 doses of extracts [groups 4, 5 and 6 (ovariectomized)]. Evaluation of weight focused on uterus and aorta, biochemical evaluation focused on serum and aorta supernatant and histological evaluation focused on vagina and uterus. Data were assessed using one-way analysis of variance (ANOVA) and *post hoc* Tukey's test. **Results:** No behavioral abnormality was observed in rats treated with extract in comparison with normal animal. In relation to sham-operated control, ovariectomy induced dystrophy of vagina and uterine, a significant increase of aorta wet weight, total cholesterol, triglycerides, LDL-cholesterol and malondialdehyde levels (in aorta) as well as an important decrease of HDL-cholesterol and nitrites levels (in aorta). Treatment with plant extract as well with estradiol induced differentiation of vaginal cornification in ovariectomized rats and increased vaginal epithelial cell, height of vagina and uterine epithelia. Furthermore, *Anthocleista schweinfurthii* improved lipid profile and oxidative stress status in aorta. **Conclusion:** *Anthocleista schweinfurthii* had low toxicity and shown estrogenic activity in ovariectomized rats by reducing certain post-menopausal symptoms.

Key words: *Anthocleista schweinfurthii*, acute toxicity, estrogenic activity, post-menopausal symptoms, malondialdehyde

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reproductive aging resulting in menopause is characterized by the permanent cessation of ovarian follicular activity. The signs and symptoms resulting from estrogen withdrawal can significantly disrupt women's activities of daily living and sense of well-being[1]. To alleviate these problems, since decades, hormone replacement therapy (HRT) has been successfully used, although large clinical trials have mentioned serious wrong side effects[2]. So, women preferred to use various forms of alternative therapies to treat menopausal symptoms[3]. Recently, plant based compounds with estrogenic effects, known as phytoestrogens, have come to the lime light[4]. Phytoestrogens can be used for the management of menopausal symptoms with few wrong side effects[5]. Epidemiological data show that a diet rich in phytoestrogens, such as those found in soy, reduce hot flashes and the incidence of cancer in Oriental women[6]. Although phytoestrogens display estrogenic effects, they are not as potent as synthetic estrogens in their estrogenic actions and are therefore considered to be safer alternatives. Consequently, researchers are looking for phytoestrogens which would be useful in preventing several symptoms of menopause and therefore, improving the quality of life of menopausal women without the risk associated with HRT[7]. *Anthocleista schweinfurthii* (*A. schweinfurthii*) is a shrub of secondary forests. Stem bark decoction is used empirically in the treatment of female infertility, hernia and ovarian problems[8]. This plant is also used to treat classic menopause symptoms. However, within the limits of knowledge till date, it does not have any scientific data on efficiency of this plant on reproductive function of female reproduction. The purpose of this study was to evaluate the degree of toxicity of *A. schweinfurthii* and to investigate the potential estrogenic activities of the extract of the same plant in some post-menopausal symptoms.

MATERIALS AND METHODS

The study was carried out in the Animal House and the Animal Physiology Laboratory of the University of Yaoundé I, Cameroon from December, 2014-April, 2015.

Plant material: Stem bark of *A. schweinfurthii* was collected at Foumban, Western region of Cameroon, in July, 2014. The voucher specimen was authenticated by Pr. Zapfack Louis, Botanist (systematic/ecology), Department of Vegetal Biology, University of Yaoundé I, Cameroon. The sample of the plant was identified at the National herbarium in comparison to the voucher number 9890SRF/Cam.

Extraction of plant material: The stem bark was washed with tap water, cut in small pieces and dried at ambient temperature. Dried pieces of the stem bark of *A. schweinfurthii* was pulverized and 160 g were dissolved in 4 L of tap water. The mixture was boiled 45 min. Decoction obtained was decanted, filtered with Watmann No. 3 filter paper and the resulting filtrate was evaporated at 45°C in oven. The yield was 11.49%. The extract obtained was diluted separately with distilled water to give doses required for each experiment and kept at 4°C.

Animals: Females Wistar rats aged of 3 months approximately, weighting about 150 g were obtained from the Animal House of the Laboratory of Animal Physiology of the University of Yaoundé I. Rats were housed in plastic cages at room temperature under natural day/night cycle. They had free access to tap water and commercial food pellets devoid of soya. All the experimental protocol was undertaken in accordance with the guidelines established by the European Union on Animal Care (CEE Council 86/609) adopted by the Ethical Committee of the Cameroonian Ministry of Scientific Research and Technology Innovation (Reg. No. FWA-IRD 0001954).

Experimental design

Acute toxicity study: Three adult female and three adult male rats were administrated orally a single dose of 2000 mg kg^{-1} b.wt., using an intragastric cannula. The control group (consisting of 6 rats) was also divided like test group and received distilled water. The maximum volume administered did not exceed 1 mL/100 g of body weight. Animals were observed individually at least once during the first 30 min after treatment gavage, periodically during the first 24 h and everyday thereafter, for a total of 14 consecutive days for behavioural changes and mortality[9].

Post-menopause induction: Thirty adult rats were ovariectomized bilaterally or sham operated under anesthesia with diazepam (10 mg kg^{-1}, i.p) and ketamine (50 mg kg^{-1}, i.p) according to modified method of Sotiriadou *et al.*[10]. Ovariectomy was preceded by a midline dorsal skin incision. After peritoneal cavity was accessed through two small dorsal incisions between the iliac crest and the lower ribs, the ovary

was found, surrounded by a variable amount of fat. After careful exposure of the ovaries, they were clamped between two mosquito clamps in order to prevent bleeding and removed after ligation of the surrounding tissue. Muscle and skin incisions were closed with a single stitch and treated with penicillin ointment to prevent infection for a week. Eighty four days after surgery, all rats were randomly divided into six groups of five animals.

Animal's treatment: Three groups were assigned to receive three different preparation of the extract plant: High dose 400 mg kg^{-1}/day (AS 3), middle dose 300 mg kg^{-1}/day (AS 2) and low dose 200 mg kg^{-1}/day (AS 1) based on body weight. Estradiol valerate (E_2) was given on 1 mg kg^{-1}/day/b.wt., for positive control. Distilled water was used like the vehicle of the extract and E_2V and used for the negative control (OVX) and sham operated group at the dose of 10 mL kg^{-1} day. Administration was made by gastric gavage for 28 days. At end of the experiment, vaginal smears were carrying out and then, animals were anesthetized and sacrificed. After sacrifice, blood samples were collected and were centrifuged at 3000 rpm for 15 min. Aorta, vagina and uterus were carefully removed, cleaned and weighed (only aorta and uterus). Aorta was homogenized at 10% in McEven solution and centrifuged at 3000 rpm for 15 min. The serum and homogenate were kept at-20°C for some biochemical analysis. Uteri and vagina were fixed in Bouin's fluid for histology.

The relative organ wet weight of each organ or organ body weight ratio was calculated using the following formula according to Akhtar *et al.* [11]:

$$\text{Organ weight ratio} = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100$$

Vaginal smears: Smears were taken from rats by flushing the vagina with 20 µL of 0.9% NaCl in a micropipette before sacrifice. A drop of smear was deposited onto a glass slide and vaginal cells were observed using a light microscope. Animals with flat fully cornified cells were defined as being in estrus. Thereafter, glass slide was dried at room temperature and stained according to the method of Papanicolaou[12].

Biochemical analysis: Serum total cholesterol, HDL-cholesterol and triglycerides were determined using a commercial diagnostic kit from Fortress diagnostics (Antrim Technology Park-United Kingdom). Levels of LDL-cholesterol were estimated from total-cholesterol, HDL-cholesterol and triglycerides by using the formula of Friedewald *et al.*[13].

Total protein levels were determined in uterus using colorimetric methods described by Gornall *et al.*[14].

Oxidative stress parameters investigation: Malondialdehyde (MDA) in aorta homogenate was determined using the procedure described by Wilbur [15] while the nitrites content was determined using the method describe by Green *et al.*[16].

Histomorphometry: Histomorphometry was carried out on 5 µm sections of vaginal and uterine epitheliums. Measures were made on the sections with a microscope (Olympus) connected to a computer and a digital camera for microscope (DCM35 350K pixels). The uterine and vaginal epithelium thicknesses were measured on 5 slides/group and the software Image J 1.32j version, NIH, California, USA were used for measurements. Histology process begins by dehydration of vagina and uterus in ascending grade of alcohol. The tissues were then cleared with xylene to remove the alcohol. Thereafter, embedding in paraffin with stainless steel block was done and sectioning was carried out using a microtome. Tissues were deparaffined in successive baths of xylene and absolute alcohol and rehydrated before staining in haematoxylin/eosin. Stained sections were observed under microscope for the microarchitectural changes.

Statistical analysis: Values were expressed as the mean±SEM. Data were assessed using one-way analysis of variance (ANOVA) and *post hoc* Tukey's test. Software used was GraphPad Prism 5.03 version, La Jolla, USA. Results were considered significant at $p<0.05$.

RESULTS

Acute toxicity: Fourteen days after the treatment, no lethality or behavioral changes were recorded in rats following single administration of aqueous extract of *A. schweinfurthii* stem bark at oral dose of 2000 mg kg^{-1} b.wt.

Effects on the vaginal smears and epithelium: The graphic representation (Fig. 1) shows the results of vaginal cytology and vaginal epithelial height after 28 days trial with our plant extract. As expected, ovariectomy induced the proliferation of parabasal cells on vaginal smears, sign of diestrus and atrophy of vaginal epithelium due to depletion of estrogen level in comparison with sham-operated group. Administration of E_2 induced significant increase ($p<0.001$) of the height of vaginal epithelium and persistent estrus

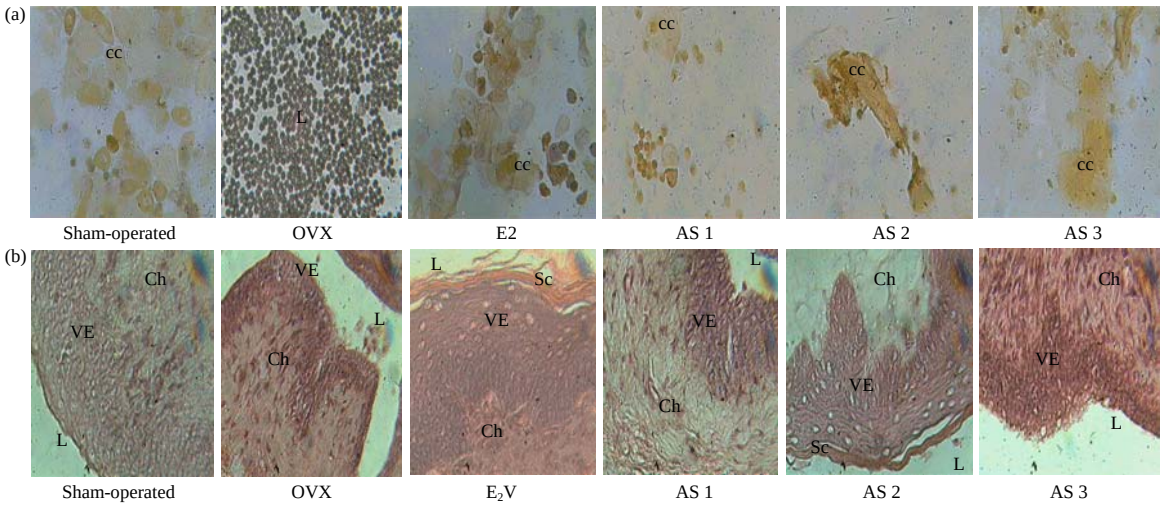

Fig. 1: Effects of *A. schweinfurthii* and E_2 on vaginal cytology (a) (Papanicolaou coloration, x 400) and on vaginal epithelium (b) (HE, x400) after 28 days trial. Cornified cells (CC), leukocytes (L), Stratum corneum (Sc), Vaginal epithelium (VE), Lumen (L), Chorion (Ch)

endocrine status. This status was reflected by appearance of cornified epithelial cells smears from OVX within 28 days relative to negative control (Fig. 1a and 1b). Some cornified cells were observed following exposure to all the doses of *A. schweinfurthii* in vaginal signs of. In the other hand, all doses of *A. schweinfurthii* induced a significant increase ($p<0.001$) of height of vaginal epithelium versus OVX animals. (Fig. 1b).

Uterotrophic bioassay: As shown in Fig. 2a, uterine wet weight decreased significantly ($p<0.001$) in OVX rats treated with distilled water compared to sham-operated rats while treatment of OVX rats with E_2 increased significantly ($p<0.001$) uterine wet weight as compared to OVX rats treated with distilled water. There was no significant increase of uterine wet weight observed between extracts treated groups and their control receiving vehicle but *Anthocleista schweinfurthii* at all doses increased uterine wet weight respectively by 32.16, 8.40 and 6.29% comparatively to OVX rats treated with distilled water. Ovariectomy decreased uterine total protein levels by 20.55% in comparison with sham operated control. *A. schweinfurthii* at dose of 400 mg kg^{-1} induced statistically significant ($p<0.01$) increase of uterine total protein levels (Fig. 2b). In the other hand, all doses of *A. schweinfurthii* increased significantly the height of uterine epithelium ($p<0.001$, $p<0.05$) (Fig. 2).

Effects of *Anthocleista schweinfurthii* on blood lipid profiles: The OVX group showed an important increase of fasting serum triglycerides levels ($p<0.001$), of fasting serum total cholesterol levels ($p<0.01$, 60.83%) and serum LDL-cholesterol levels ($p<0.01$) compared to sham-operated animals, while the HDL-cholesterol levels tended to decrease (48.18%). This has also led to the increase ($p<0.01$) of the atherogenic index calculated as the ratio of total cholesterol on HDL-cholesterol, due to the loss of ovarian estrogens. E_2 (1 mg kg^{-1} b.wt.,) and the aqueous extract of *A. schweinfurthii* at all doses have significantly decreased fasting serum triglycerides and the atherogenic index. Only the dose of 200 mg kg^{-1} significantly decreased respectively total cholesterol and LDL-cholesterol levels after 28 days of treatment compared to OVX group. On the other hand, serum HDL-cholesterol levels of rats treated with aqueous extract plant at the doses of 200 and 300 mg kg^{-1} b.wt., were significantly increased (Table 1).

Effects of *Anthocleista schweinfurthii* on aorta: The results of the present study showed respectively that (Fig. 3 a, b and c) 112 days of estrogenic deprivation significantly increased aorta weight ($p<0.05$), MDA levels ($p<0.01$) and decreased nitrites levels (58.52%) as compared to sham control aorta. The same graphic representations show significant decrease of aorta weight after sub-chronic treatment with E_2 ($p<0.01$) and with plant extract at all doses (respectively $p<0.01$; $p<0.001$ and $p<0.001$) versus OVX group. *A. Schweinfurthii* at the dose of 400 mg kg^{-1} decreased significantly ($p<0.001$) MDA levels versus OVX group while both E_2 and all doses of our plant extract increased significantly nitrites levels compared to OVX group.

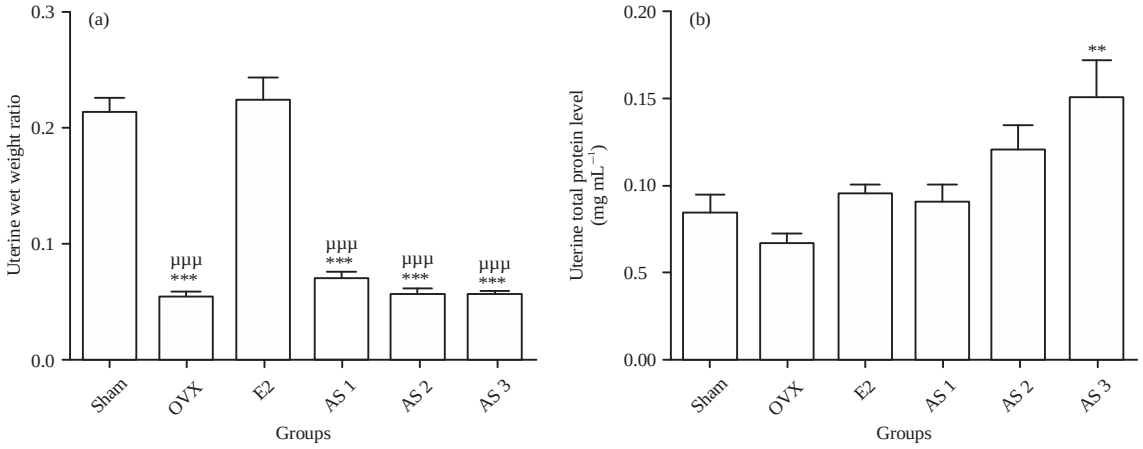

Fig. 2: Effects of *A. schweinfurthii* and E_2 on (a) uterus wet weight and on (b) uterine total protein levels after 28 days treatment on ovariectomized rats

μμμ p<0.001 statistically significant compared OVX with sham-operated. ***p<0.001 significant compared all doses with E_2, ** p<0.01 statistically significant compared AS 3 with OVX

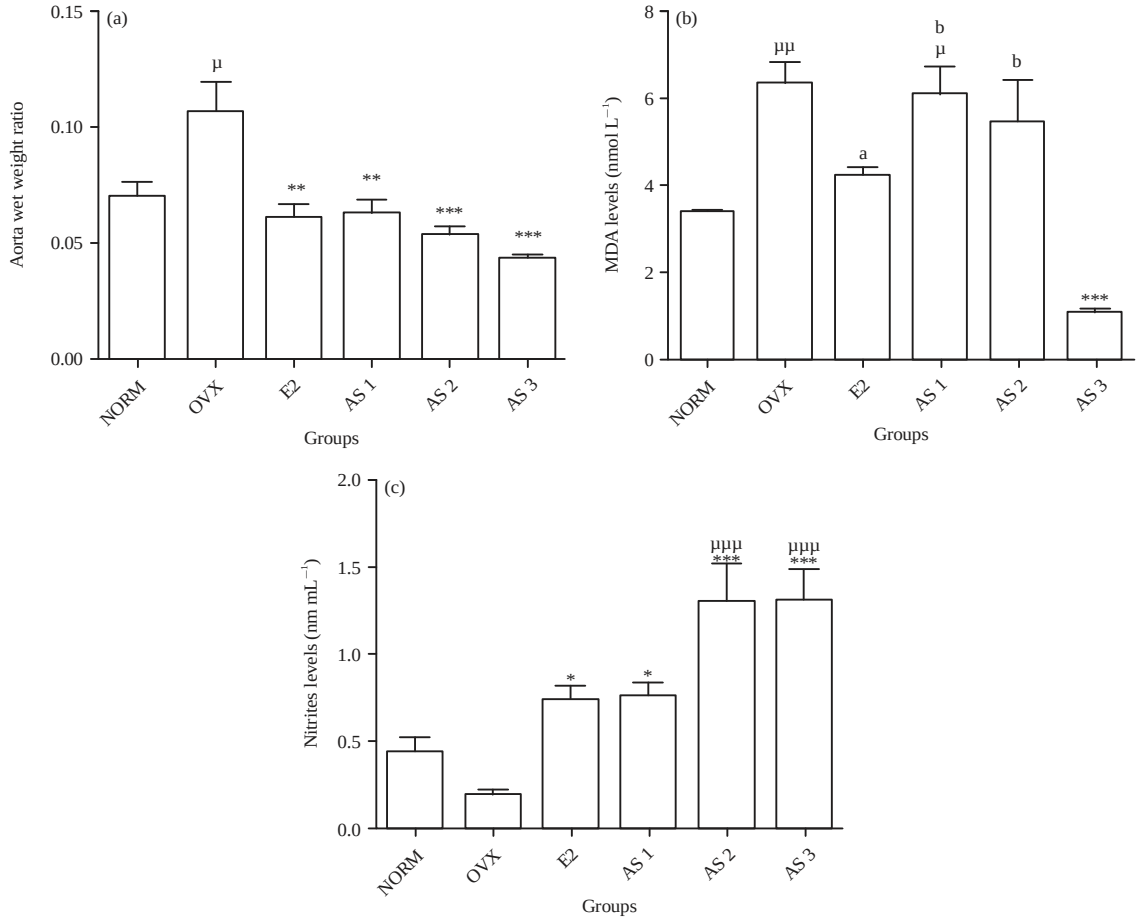

Fig. 3: Effects of *A. schweinfurthii* and E_2 on (a) aorta wet weight, (b) malondialdehyde and (c) nitrites after 28 days treatment on ovariectomized rats

μ p<0.05, μμ p<0.01, μμμ p<0.001 statistically significant-compared with sham-operated. **p<0.01, ***p<0.001, significant compared all doses with OVX, a p<0.01 and b p<0.001 significant compared with AS 3. (n = 5/group)

Table 1: Effects of *A. schweinfurthii* and E_2 on blood lipid profiles after 28 days of treatment

	Groups					
Parameters	Sham-operated	OVX	E_2	AS 1	AS 2	AS 3
Triglycerides levels (mg dL^{-1})	51.12±4.80	103.30±12.49###	61.5±7.78**	45.59±4.42***	61.84±1.97**	68.07±3.95*
Total cholesterol (mg dL^{-1})	46.32±4.97	93.92±2.85##	65.75±5.86	56.81±7.90*	63.67±4.47	64.93±10.01
LDL-cholesterol (mg dL^{-1})	33.09±4.80	71.04±9.26##	50.2±0.90	34.70±3.51**	44.08±5.32	39.95±6.27*
HDL-cholesterol (mg dL^{-1})	2.84±0.13	1.47±0.16	2.61±0.33	5.370±0.80#, ***	4.68±0.34#, ***	3.19±0.06 a
Atherogenic index	18.46±2.09	61.95±14.18##	27.50±2.11*	11.33±2.11***	13.99±1.99***	20.32±3.11**

Values are presented as means±SEM. ### p<0.001, ## p<0.01, # p<0.05 statistically significant compared with sham-operated, ***p<0.001, **p<0.01, *p<0.05 statistically significant compared with OVX, a p<0.05 statistically significant compared AS 1 versus AS 3: The extracts of *A. schweinfurthii* stem bark induced significant dose dependent HDL-cholesterol levels in OVX rats. (n=5/group)

DISCUSSION

In the present study, the evaluation of acute oral administration of a single dose of 2000 mg kg^{-1} of the aqueous extract of *A. schweinfurthii* indicated that this plant did not induced any mortality or change of behaviour in female and male young Wistar albino rats. According to the recommendations of OECD guideline 423[9], any substance can be considered as low toxic if its LD_{50} is greater than 2000 mg kg^{-1}. So our extract can be considered as low toxic because administration of 2000 mg kg^{-1} did not induced any mortality or change of behaviour. In fact, herbal medicines have received greater attention as alternative to clinical therapy of many diseases and the demand for these remedies has currently increased[17]. Because many published studies have reported potential toxic effects of natural products, it is necessary to characterize the effects of plant on biological systems, including its toxicological effects.

Study of the estrogenic effects of plant extracts can be realized on estrogenic organs target such as uterus and vagina. Vagina is a musculo-membranous channel formed of a non-keratinized stratified squamous epithelium. Vaginal smears allow the determination vaginal epithelial cells status[18]. The duration of estrus cycle in rats is normally 4-5 days. During a normal rat estrus cycle, there are three cell types: Parabasal, intermediate and cornified cells. Presence or absence of these types of cells and their relative proportions determine the stage of estrus cycles phase. Estrus cycle is controlled by the synthesis of estrogen in the ovary[19]. Absence of cornified cells and affluent proportion of parabasal cells observed on vaginal smear of OVX group in this study is due to estrogen deficiency caused by ovariectomy. Oral administration of *A. schweinfurthii* allowed apparition of intermediate and cornified cells indicating that treated animals were in the estrus phase and a significant increase of the vaginal epithelial height, proof of his estrogen like activity. The same results were obtained with another suspected phytoestrogen, *Citrus medica* in ovariectomized rats by El-Alfy *et al.*[19].

Uterus is a primary target organ for estrogen. The uterotrophic assay is a standard tool for determining the estrogenic activity of a given substance *in vivo*[20]. The investigation has shown a small but remarkable increase of the uterine wet weight, uterine total protein levels and stimulation of the height of the uterine epithelium after oral administration of *A. schweinfurthii* during 28 days. These results are consistent with Diel *et al.*[21], who showed that certain phytoestrogens in contrast to estrogens, failed to stimulate increased tumour growth or estrogen-sensitive gene expression. The data have to be interpreted in favour of mechanistic differences in the action of *A. schweinfurthii* as compared to other compounds with affinity to the estrogens receptors like genistein[22].These data could demonstrate that the molecular mechanisms involved in the uterine activity of *A. schweinfurthii* are very complex and may be distinct from those of endogenous estrogens.

Ovariectomy increased triglycerides; total cholesterol, LDL-cholesterol and artherogenic index and decreased HDL-cholesterol in serum after 112 days. The data are in accordance the study of Lj *et al.* [23], who prove that women in physiological menopause have much higher levels of total cholesterol, LDL cholesterol, triglycerides and lower levels of HDL-cholesterol. These variations may be responsible for cardiovascular diseases. Indeed, the decrease in endogenous estradiol as a result of ovariectomy may affect lipoprotein concentrations[24] like lipoprotein (a) which has been found to be a new independent factor associated with cardiovascular diseases[25]. In fact, several studies have shown a significant rising in lipoprotein (a) levels with menopause, as well as the risk of cardiovascular diseases independently of LDL levels[26-27]. It is likely that age plays a significant role in developing dyslipidemia. Furthermore, according to Park *et al.*[28], decrease of the synthesis of estrogens stimulates lipoprotein lipase which hydrolyses triglycerides and consequence is accumulation of fatty acids, responsible of cardiovascular diseases. The data can also be explained by the fact that depletion of estrogen inhibits cholesterol 7α-hydroxylase.

Deficiency in this enzyme leads to increase cholesterol biosynthesis in liver but also to stimulate expression of various genes involved in biosynthesis of associated hypertriglyceridemia[29]. Treatment with plant extract and E_2 decreased significantly triglycerides, total cholesterol, LDL-cholesterol, artherogenic index and increased HDL-cholesterol. This beneficial effect of *A. schweinfurthii* on lipid metabolism may be due to its ability to activate estrogen receptors and induce the transcription of genes involved in lipid metabolism. Therefore, *A. schweinfurthii* may be used like *Pueraria mirifica*, a Thai herb rich in phytoestrogens with a beneficial effect on lipid metabolism in postmenopausal women, which may result from the activation of gene transcription through selective binding of phytoestrogens to ER α and ER β[30].

Ovariectomy increased significantly the weight of aorta as well as the level of MDA in that organ, increase that was lower after sub-chronic treatment to E_2 and to plant extract at all doses. Surgery also decreased nitrites levels in aorta. All effects on aorta weight after ovariectomy can be explained by depletion of estrogen. In fact, estrogen is known to induce rapid vasodilatory response in isolated arteries[31]. The lack of that hormone causes hypercholesterolaemia (elevation of plasma total cholesterol, LDL-cholesterol and decreased HDL) who may contribute to the pathogenesis of atherosclerosis by increasing of lipid peroxidation. LDL-cholesterol in the blood moves inside the vessel wall and is then oxidized and will contribute to atherogenic process. The oxidized LDL is the sole responsible factor altering the structure and function of the endothelial cell which attract monocytes and macrophages by chemotactive action which then develop into the lipid laden foam cells of an atheromatous plaque, responsible to increasing of aorta weight[32]. Treatment reduced aorta wet weight and MDA levels but increased nitrites levels. These beneficial effects of our extracts may be related to its estrogen mimetic effects. In fact, it is well known that administration of estrogen and phytoestrogen decrease low-density lipoprotein (LDL) levels and increase high-density lipoprotein (HDL) levels in postmenopausal women[33] via the ability of those substances to exhibit antioxidant properties that are responsible for the protective action against lipid peroxidation[34]. Extract of *A. schweinfurthii* significantly increased nitrites levels compared to vehicles controls. This effect of extract may be related to the presence of secondary metabolites like flavonoids which are able to modulate vascular tone via a rapid release of endothelium release NO and up regulate eNOS expression[35].

CONCLUSION

A. schweinfurthii had low toxicity by oral route. Ovariectomy has deleterious effects on vagina, uteri, lipid parameters and on aorta. *A. schweinfurthii* like oestradiol valerate, induced vaginal and uteri epithelial thickening. It also prevented menopause associated high lipid profiles indicating that *A. schweinfurthii* may be useful in preventing some of menopausal symptoms. More study is still to be carried out in order to determine the mechanism of action of the plant extract on disturbances related to oestrogen deficiency.

SIGNIFICANCE STATEMENTS

This study discovers the low toxicity of *Anthocleista schweinfurthii* and it estrogenic effect that can be beneficial for dyslipidemia induced by ovariectomy of Wistar Rats. This study will help the researcher to uncover the critical area of postmenopausal cardiovascular disorder that many researchers were not able to explore. Thus, a new theory on these phytoestrogen and possibly others phytoestrogens, may be arrived at.

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REFERENCES

1. Sekhon, L.H. and A. Agarwal, 2013. The Menopause and Oxidative Stress. In: Studies on Women's Health, Agarwal, A., N. Aziz and B. Rizk (Eds.). Humana Press, Totowa, NJ., USA., ISBN: 978-1-62703-040-3, pp: 181-203.
2. Burger, H., 2003. Hormone replacement therapy in the post-Women's health initiative era. Climacteric: J. Int. Menopause Soc., 6: 11-36.
3. Pitkin, J., 2012. Alternative and complementary therapies for menopause. Menop. Inter., 18: 20-27.
4. Al-Anazi, A.F., V.F. Qureshi, K. Javaid and S. Qureshi, 2011. Preventive effects of phytoestrogens against postmenopausal osteoporosis as compared to the available therapeutic choices: An overview. J. Nat. Sci. Biol. Med., 2: 154-163.
5. Glazier, M.G. and M.A. Bowman, 2001. A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. Arch. Int. Med., 161: 1161-1172.

6. Kurzer, M.S. and X. Xu, 1997. Dietary phytoestrogens. Ann. Rev. Nutr., 17: 353-381.
7. Njamen, D., B.N. Nkeh-Chungag, M.A. Mvondo and N.S. Tchoukouegno, 2013. Oestrogenic properties of the ethanolic extract of *Fernandoa adolfi friderici* (Bignoniaceae) stem bark. Afr. J. Pharm. Pharmacol., 7: 1729-1736.
8. Kerharo, J., 1974. La Pharmacopee Senegalaise Traditionnelle: Plantes Medicinales et Toxiques. Edition the Book, Paris, pp: 508-510.
9. OCDE., 2001. Toxicite orale aigue, methode de la dose predeterminee. Organization for Economic Co-operation and Development (OCDE), Ligne directrice de l'OCDE Pour Les Essais de Produits Chimiques: Ligne directrice n° 423, 1-15, [In French].
10. Sotiriadou, S., A. Kyparos, V. Mougios, C.H. Trontzos, G. Sidiras and C.H. Matziari, 2003. Estrogen effect on some enzymes of female rats after downhill running. Physiol. Res., 52: 743-748.
11. Akhtar, N., M.K. Srivastava and R.B. Raizada, 2009. Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*. J. Environ. Biol., 30: 1047-1053.
12. Papanicolaou, G.N., 1942. A new procedure for staining vaginal smears. Science, 95: 438-439.
13. Friedewald, W.T., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18: 499-502.
14. Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-766.
15. Wilbur, K.M., 1949. The Thiobarbituric acid reagent as a test for the oxidation of unsaturated fatty acids by various agents. Arch. Biochem., 24: 305-313.
16. Green, L.C., D.A. Wagner, J. Glogowski, P.L. Skipper, J.S. Wishnok and S.R. Tannenbaum, 1982. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. Anal. Biochem., 126: 131-138.
17. Otimenyin, S.O., P.O. Olorunfemi, Y.S. Sabo and S.H. Attah, 2013. Sub acute toxicity studies of the herbal cough mixture (hamekof) in adult male Wister rats. J. Med. Med. Sci., 4: 439-450.
18. Ahn, E.M., N. Nakamura, T. Akao, T. Nishihara and M. Hattori, 2004. Estrogenic and antiestrogenic activities of the roots of *Moghania philippinensis* and their constituents. Biol. Pharm. Bull., 27: 548-553.
19. El-Alfy, T.S., M.H. Hetta, N.Z. Yassin, R.F. Abdel-Rahman and E.M. Kadry, 2012. Estrogenic activity of *Citrus medica* L. leaves growing in Egypt. J. Applied Pharmaceut. Sci., 2: 180-185.
20. Diel, P., R.B. Geis, A. Caldarelli, S. Schmidt, U.L. Leschowsky, A. Voss and G. Vollmer, 2004. The differential ability of the phytoestrogen genistein and of estradiol to induce uterine weight and proliferation in the rat is associated with a substance specific modulation of uterine gene expression. Mol. Cell. Endocrinol., 221: 21-32.
21. Diel, P., T. Schulz, K. Smolnikar, E. Strunck, G. Vollmer and H. Michna, 2000. Ability of xeno-and phytoestrogens to modulate expression of estrogen-sensitive genes in rat uterus: Estrogenicity profiles and uterotropic activity. J. Steroid Biochem. Mol. Biol., 73: 1-10.
22. Diel, P., S. Olff, S. Schmidt and H. Michna, 2001. Molecular identification of potential Selective Estrogen Receptor Modulator (SERM) like properties of phytoestrogens in the human breast cancer cell line MCF-7. Planta Med., 67: 510-514.
23. Lj, M., S. Vujovic, Z. Penezic, M. Stojanovic, M. Ivovic and M. Drezgic, 2006. Lipids and women in early and physiological menopause. Maturitas, 54: S49-S49.
24. Chamberlain, A.M., A.R. Folsom, P.J. Schreiner, E. Boerwinkle and C.M. Ballantyne, 2008. Low-density lipoprotein and high-density lipoprotein cholesterol levels in relation to genetic polymorphisms and menopausal status: The Atherosclerosis Risk in Communities (ARIC) study. Atherosclerosis, 200: 322-328.
25. Agacayak, E., S. Basaranoglu, S.Y. Tunc, M.S. Icen and F.M. Findik *et al*., 2015. Oxidant/antioxidant status, paraoxonase activity and lipid profile in plasma of ovariectomized rats under the influence of estrogen, estrogen combined with progesterone and genistein. Drug Design Dev. Therapy, 9: 2975-2982.
26. Carr, M.C., 2003. The emergence of the metabolic syndrome with menopause. J. Clin. Endocrinol. Metab., 88: 2404-2411.
27. Simoncig-Netjasov, A., S. Vujovic, M. Ivovic, M. Tancic-Gajic, L. Marina and M. Barac, 2013. Relationships between obesity, lipids and fasting glucose in the menopause. Srpski Arhiv za Celokupno Lekarstvo, 141: 41-47.
28. Park, H.J., M.A. Della-Fera, D.B. Hausman, S. Rayalam, S. Ambati and C.A. Baile, 2009. Genistein inhibits differentiation of primary human adipocytes. J. Nutr. Biochem., 20: 140-148.
29. Repa, J.J., E.G. Lund, J.D. Horton, E. Leitersdorf, D.W. Russell, J.M. Dietschy and S.D. Turley, 2000. Disruption of the sterol 27-hydroxylase gene in mice results in hepatomegaly and hypertriglyceridemia reversal by Cholic acid feeding. J. Biol. Chem., 275: 39685-39692.
30. Zingue, S., D. Njamen, J. Tchoumtchoua, M. Halabalaki, E. Simpson, C. Clyne and C.B.M. Nde, 2013. Effects of *Millettia macrophylla* (Fabaceae) extracts on estrogen target organs of female Wistar rat. J. Pharmacol. Sci., 123: 120-131.
31. Bolego, C., A. Cignarella, P. Sanvito, V. Pelosi, F. Pellegatta, L. Puglisi and C. Pinna, 2005. The acute estrogenic dilation of rat aorta is mediated solely by selective estrogen receptor-α agonists and is abolished by estrogen deprivation. J. Pharmacol. Exp. Ther., 313: 1203-1208.

32. Adam, S.K., S. Das and K. Jaarin, 2009. A detailed microscopic study of the changes in the aorta of experimental model of postmenopausal rats fed with repeatedly heated palm oil. Int. J. Exp. Pathol., 90: 321-327.
33. Herrington, D.M., T.D. Howard, G.A. Hawkins, D.M. Reboussin and J. Xu *et al*., 2002. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. N. Engl. J. Med., 346: 967-974.
34. Badeau, M., H. Adlercreutz, P. Kaihovaara and M.J. Tikkanen, 2005. Estrogen A-ring structure and antioxidative effect on lipoproteins. J. Steroid Biochem. Mol. Biol., 96: 271-278.
35. Joy, S., R.C. Siow, D.J. Rowlands, M. Becker and A.W. Wyatt *et al*., 2006. The isoflavone Equol mediates rapid vascular relaxation: Ca2+-independent activation of endothelial nitric-oxide synthase/Hsp90 involving ERK1/2 and Akt phosphorylation in human endothelial cells. J. Biol. Chem., 281: 27335-27345.