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Estrogenic and anxiolytic effects of the decoction of stem bark of *Khaya anthotheca* (Welw.) C.DC (Meliaceae) in ovariectomised wistar rats

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Abstract

Khaya anthotheca (Welw.) C.DC (Meliaceae) is a plant used in Cameroon to alleviate vaginal dryness in postmenopausal women and is also known to have anxiolytic properties.This work was designed to evaluate estrogen-like effects of this plant on primary estrogens targets of ovariectomized adult rats, as well as to evaluate its anxiolytic activities in the elevated plus-maze (EPM) test. In the 3-day uterotrophic assay, the extract increased ($p < 0.01$) the size of the vaginal epithelia and stimulated the acini differentiation of the mammary gland. In the EPM test, the extract increased the percentage of number of entries ($p < 0.05$; $p < 0.01$) and the percentage of time spent ($p < 0.05$) into open arms. It also induced a decrease in percentage of number of entries ($p < 0.05$; $p < 0.01$) and the percentage of time spent ($p < 0.05$) into closed arms. The extract also induced an increase of total arms entries ($p < 0.05$; $p < 0.01$) and rearing ($p < 0.05$). Moreover, there was a decrease of defecation and grooming ($p < 0.05$; $p < 0.01$). These results suggest that *K. anthotheca* is endowed with estrogenic and anxiolytic properties, likely due to the presence of some estrogen-like compounds.

Keywords: *Khaya anthotheca*, ovariectomy, estrogenic properties, anxiolytic, elevated plus-maze.

Introduction

Menopause marks the end of the reproductive life span of women and is characterized by a dramatic drop in circulating estrogens. Symptoms associated with this estrogen deprivation include loss of libido, vasomotor instability (hot flushes), insomnia, depression, anxiety and vaginal dryness [1,2] Diseases of the nervous system such as anxiety are always the most invalidating of all the diseases assigning the Man. Statistically, between 10 % of the world population suffer from several forms of anxiety [3] and 20 % of the adult population will suffer from this pathology at least oncein its life because it is expected that from 2001 to 2026, the world population aged of 65 years old and more will pass from 550 to 973 million [4], since there is a correlation between aging and risk of anxiety. These make anxiety to be considered as one of the principal objectives of psychopharmacology researches in this last decade. Within menopausal women, this pathology is accentuated by the deficiency in circulating estrogen and approximately 10 % to 25 % of women suffering from such diseases affecting the nervous system seek a treatment [2].

The first approach of treatment is the use of benzodiazepines. However, these anxiolytic and sedative drugs that modulate GABAA receptors have many secondary effects such as muscle relaxation and sedation. After medium- to long-term use, benzodiazepines produce physical dependence, tolerance, ataxia, and memory impairment [5]. Additionally, these psychotropic agents used are not appropriate for the primary treatment of symptoms due to a lack of estrogens [6]. The hormone replacement therapy (HRT) remains the principle mode of treatment of physiological problems observed in menopausal women [7]. Despite the effectiveness of hormone replacement therapy to alleviate menopause symptoms, many women refuse or discontinue treatment because of the adverse effects such as an increased risk of cancer and cardiovascular diseases [8,9,10,11]. Thus many women are increasingly turning to natural health remedies as alternative therapies to treat menopausal symptoms [12]. Consequently, this search for treatment has increased among the scientific community the need for developing new alternative treatments for the management of the physiological disorders related to the menopause [2,13].

Many studies then focused on phytoestrogens, which are plant-derived compounds with estrogenic properties. These chemicals

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are considered to be chemically and structurally analog to mammalian estrogen 17β-estradiol, which enable them to bind both estrogen receptor sub-types: estrogen receptor (ER) and estrogen receptor β (ERβ) [14]; and thus mimic estrogenic actions in mammals.

In most developing countries, the vast majority (80%) of the population utilize traditional medicine for their primary health care [3]. Even in western countries, the search for alternative medication based on plant extracts is increasing at least among menopausal and postmenopausal women [9,11]. *Khaya anthotheca* (Welw.) C.DC is a plant of the family of Meliaceae. This plant is distributed in the west, the center and the south of Africa. In African traditional medicine, this plant is used to treat diseases like helmenthiasis, malaria, gonorrhea and abdominal pain [15, 16]. The Khaya genus is also used for the treatment of convulsion, fever, cough, stomach ache, rheumatism and dermatomycosis [17]. In Cameroon, ethno botanical enquiries have revealed that this plant has anxiolytic properties and is used to alleviate vaginal dryness in postmenopausal women. Documented evidence of pharmacological activity revealed anti-protozoa [18], antihelminthic [19]. The genus shows an antioxidant activity in pharmacological study [20]. The phytochemical characterization of *K. anthotheca* revealed the presence of alkaloids [21]. Carbohydrates, saponins, trite pens, tannins, phlobatanins, steroids, flavonoids, polyphenols and anthraquinones were also found from this genus[17, 20]. In the literature, no previous study has reported estrogenic and behavioral activities of *K. anthotheca*. For the above mentioned reasons, we therefore aimed to evaluate the estrogenic and anxiolytic effects of the decoction of *K. anthotheca* in ovariectomised rats.

Material and methods

Animals

Juvenile female Wistar rats, 150 ± 10 g, aged between 10 and 12 weeks were used for this study. The animals were housed in an environmentally controlled room (temperature 25 C; humidity 50-80%; 12 hlight–dark cycle). They had free access to a standard soy-free rat diet (SSniff GmbH, Soest, Germany) and were provided tap water ad libitum. All animals' husbandry handling conditions were in accordance with the guidelines of the institutional Ethic Committee of Cameroon's Ministry of Scientific Research and Technological Innovation, which has equally adopted the guidelines established by the European Union on Animal Care (CEE Council 86/609; Reg.no.FWA-IRD0001954)

Plant material and extraction

The stem barks of *Khaya anthotheca* were collected in Mamougnam (District of Massangam, Department of Noun, West Region of Cameroun). This botanical sample was identified at the National Herbarium of Cameroon (HNC) by comparison to the specimens deposited under the voucher number 4230/HNC. After drying and grinding the stem bark, 250 g of crushed bark were carried to ebullition in 5 L of tap water for 30 min. The supernatant was collected and filtered with What man No.4 filter paper. The filtrate was lyophilized resulting in 26.87 g of the dried extract (Slightly brown powder), representing 10.75 % yield.

Chemicals

Diazepam (Valium®10mg/2ml, laboratoire Roche, Fontenay-sous-bois, France) and estradiol valerate (Progynova® 2mg, DEL-PHARM, Lille, France) was used as reference drug.

Experimental design

Before each test, all female Wistar rats were ovariectomised except the Sham rats. The bilateral ovariectomy (OVX) using the dorsal approach [22] under diazepam and ketamin anesthesia (respectively 10mg/kg and 50mg/kg BW; i.p.) was made. After 14 days of endogenous hormonal decline [23], animals were randomly distributed into groups for the tests. Estradiol vale rate, diazepam and the aqueous extract of *Khaya anthotheca* were dissolved in distilled water used as vehicle in these experiments. The doses of administration were prepared based on the traditional dosage; the equivalent doses in rat were extrapolated from the human dose (80 mg/kg BW) to afford 500mg/kg BW. To obtain a dose response curve of the extract, 3 other doses (125,250 and 1000 mg/kg BW) were generated.

The 3-day uterotrophic assay

Thirty ovariectomised rats were distributed into six groups of five animals each. The first group or OVX group received vehicle only (distilled water) and the second group received estradiol vale rate (E2V) as standard drug at the optimal dose of 1mg/kgBW per day. The remaining four groups received the aqueous extract of *K. anthotheca* (KA) at the doses of125, 250, 500 and 1000 mg/kgBW per day. All treatment was given by gavages (p.o., 2ml/100g) for 3 days. Twenty four hours after the last administration, animals were sacrificed by decapitation. The uterine wet weight, uterine and vaginal epithelial thickness and mammary gland were assessed as described before by Njamen *et al.* [24] and Zingue *et al.* [25].

Histological analysis

Using the complete Zeiss equipment consisting of a microscope (Axioskop 40) connected to a computer where the image (400X)was transferred and analyzed with the MRGrab1.0 and Axio Vision 3.1 software, all provided by Zeiss (Hallbermoos, Germany) [24]; the histomorphology of the mammary glands, as well as the

uterine and vaginal epithelial heights, were assessed from 5-µm sections of paraffin-embedded tissues following hematoxylin–eosin staining.

The elevated plus-maze test

Elevated plus maze is the simplest apparatus to study anxiolytic response of almost all types of anti-anxiety agents. It produced a novel environment which helped in inducing anxiety in animals because of the open nature of the arms and elevation from the floor. The maze consisted of two opposite open arms (50 cm 15 cm), crossed with two enclosed arms of the same dimensions with walls 50 cm high. The arms were connected with a central square, 15 cm 15 cm to give the apparatus a plus sign appearance. The maze was elevated 71 cm above the floor in a dimly lit room. Rodents have a natural aversion for high and open spaces and prefer enclosed arms, which have a burrow like ambience and therefore spend greater amount of time in the enclosed arm. When exposed to the novel maze alley, the animals experience an approach-avoidance conflict, which is stronger in the open arm as compared to the enclosed arms.

The animals were divided into eight groups of eight animals each: the NOVX group (Sham operated received the vehicle, p.o.), OVX group (received the vehicle, p.o.), 17β-estradiol group (1 mg/kg, p.o.), diazepam group (1 mg/kg, i.p.) and four groups that were given the aqueous extract (125, 250, 500 and 1000 mg/kg, p.o.). One hour following the administration of different substances (2 ml/100 g for oral administration), each rat was placed individually at the corner of an open arm and observed for a period of 5 min [26, 27, 28, 29]. The data were collected with a video-camera system and the parameters noted were time spent in open/closed arms, number of entries in the open/closed arms, total arms entries, number of rearing, number of defecation and number of grooming [30,31,32]. The percentages of times spent and the number of entries in each type of arms were calculated for each animal. To avoid perturbation of the animals due to urine and faeces, between two tests, the maze was cleaned with 70% ethanol solution and dry cloth. After the test, the rectal temperature of each animal was measured using a thermometer.

Statistical analysis

The data from each experimental group were expressed as the mean ± S.E.M. The significance of the difference between treated groups and OVX group or Shamgroupwas determined using one-way ANOVA followed by Dennett's test and the significance of the difference between OVX group and NOVX group was determined using the unpaired t-test (Graph Pad Prism, version 5.03). A p-value < 0.05 was considered significant.

Results

Results of the 3-day treatment with *K. anthotheca* extract

Effects of *Khaya anthotheca* extract on the uterine wet weight and uterine epithelium

As shown in Figure. 1 A, B and C, the 3 days oral administration of the *K. anthotheca* extract did not induce any significant effect in the uterine wet weight and uterine epithelial thickness at all tested doses as compared to the vehicle treated group (OVX). The E2V-treated group at the dose of 1 mg/kg showed a significant increase in the uterine wet weight and uterine epithelial thickness ($p < 0,001$). This increase was 4 and 3 times the value of the vehicle treated group respectively for the uterine wet weight and uterine epithelial thickness. These effects were materialized in histological sections by the formation of a tall cuboidal to columnar epithelium containing large cells following E2V treatment while in the OVX group uteri consisted of a low cuboidal epithelium(Figure. 1C).

Effects of *K. anthotheca* extract on the vaginal epithelium

3-day treatment with the aqueous extract of *K. anthotheca* at all tested doses, induced an increase of vaginal epithelial height (figure. 2 A and B). This was significant at the doses of 250, 500 and 1000 mg/kg BW ($P < 0.01$) and was respectively 77.90 %, 77.90 % and 85.40 % higher than OVX group. The graphic representation of the vaginal epithelial height (Figure. 2A) shows that the response of the extract was however less pronounced than that of E2V ($p < 0.001$). Regarding vaginal epithelial thickness (Figure. 2B), the microphotographs of vaginal epithelium of the OVX group consisted simply of the stratum germinativum (Ge) at the lowest level of the thickness. After the treatment with the extract (500 and 1000 mg/kg) and E2V (1mg/kgBW), the vaginal epithelium became stratified (Gr).

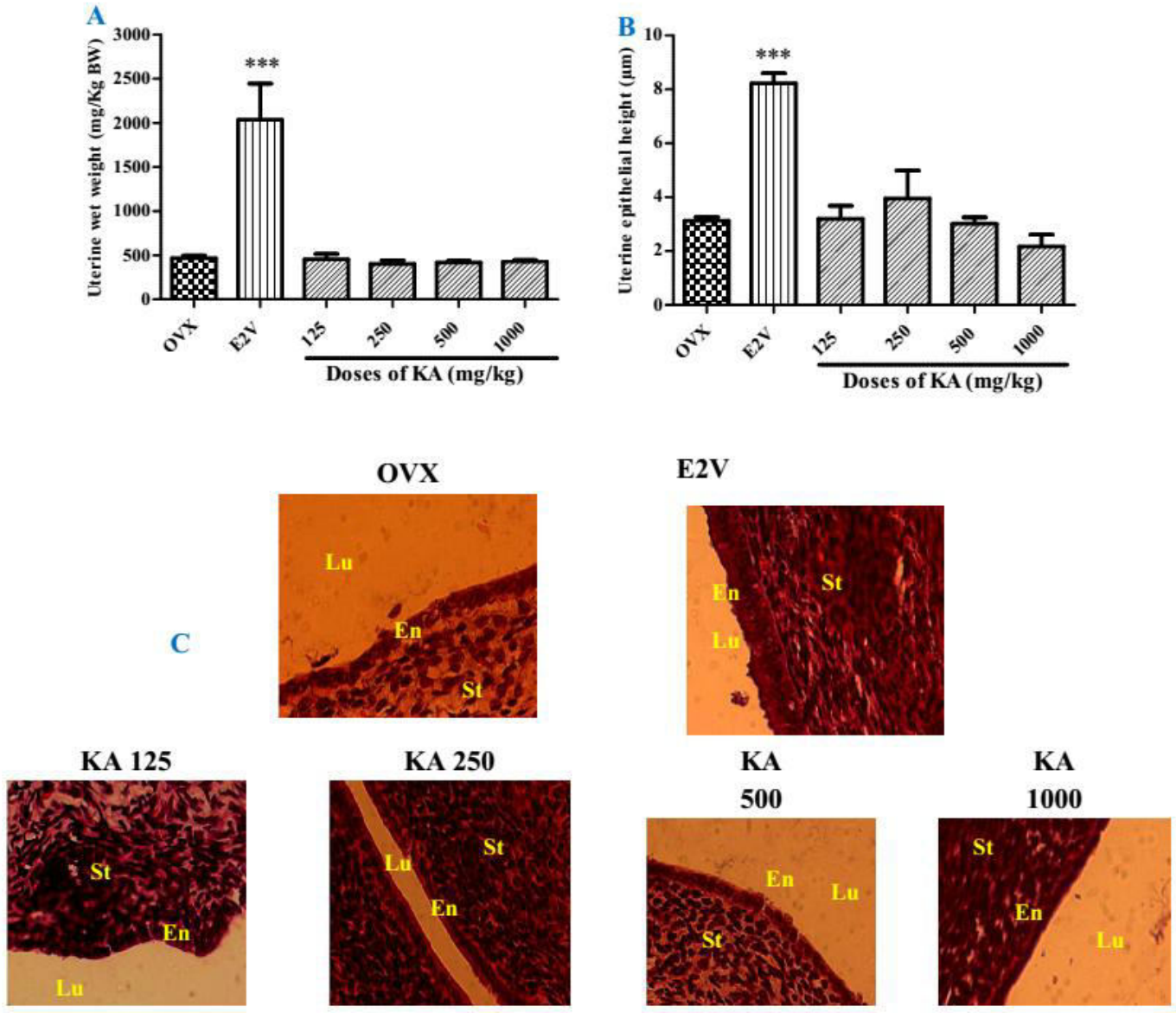

Figure 1: Effects of 3-day treatment with *Khaya anthotheca* extract on the uterine wet weight and uterine epithelial thickness. Bars represent the uterine wet weight (A) and uterine epithelial thickness (B). Data are expressed as mean ± SEM, n = 5 per group; microphotographs (C). OVX = OVX animals treated with the vehicle, E2V = OVX animals treated with estradiol vale rate at 1 mg/kg BW, KA = OVX animals treated with the aqueous extract of *K. anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg BW. ***P < 0.001vs. OVX(one-way ANOVA followed by Dunnett's test).Lu: uterine lumen; En: Endometrium; St: Stroma.

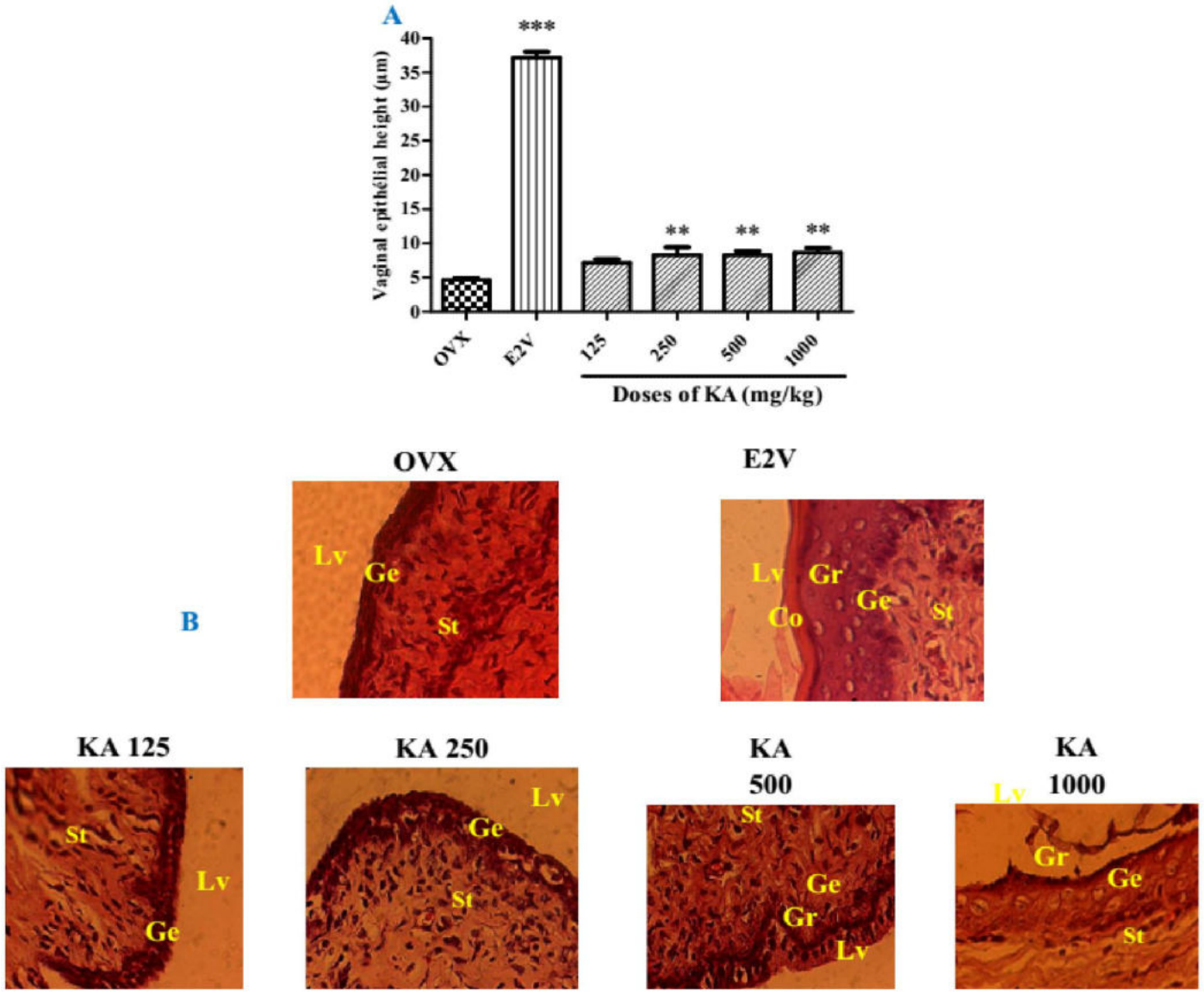

Figure 2:effects of 3-day treatment with *khaya anthotheca* extract on the vaginal epithelial thickness. Bars represent the epithelial height (a). Data are expressed as mean ± sem, n = 5 per group; microphotographs (b). Ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol valerate at 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark extract of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. $^{**}p < 0.01$,$^{***}p < 0.001$ vs. Ovx(one-way anova followed by dunnett's test).lv = vaginal lumen, co = stratum corneum, gr =stratum granulosum, ge = stratum germinativum, st = stroma.

Effects of *K. anthotheca* extract on mammary gland

Figure. 3 depicts the 5-µm sections of paraffin-embedded tissues and hematoxylin-eosin staining of mammary glands. Ovariectomy induced an atrophy of mammary gland which is materialized in OVX-histological section by a modest alveolar development, the loss of the gland parenchyma (Tc) and the ductular and alveolar components, while adipocyte tissue (At) appears prominent. This atrophy is evident by epithelial cells of alveoli, which are low cuboidal. Mammary glands of E2V-treated group depict an increase in proliferative activity compared to OVX group such as increase of the diameter and the lumen of alveoli, and abundant eosinophil secretion (Se) in lumen of alveoli. Similar changes were noticed at all tested doses withaqueous extract of *K. anthotheca*aftera3-day treatment.

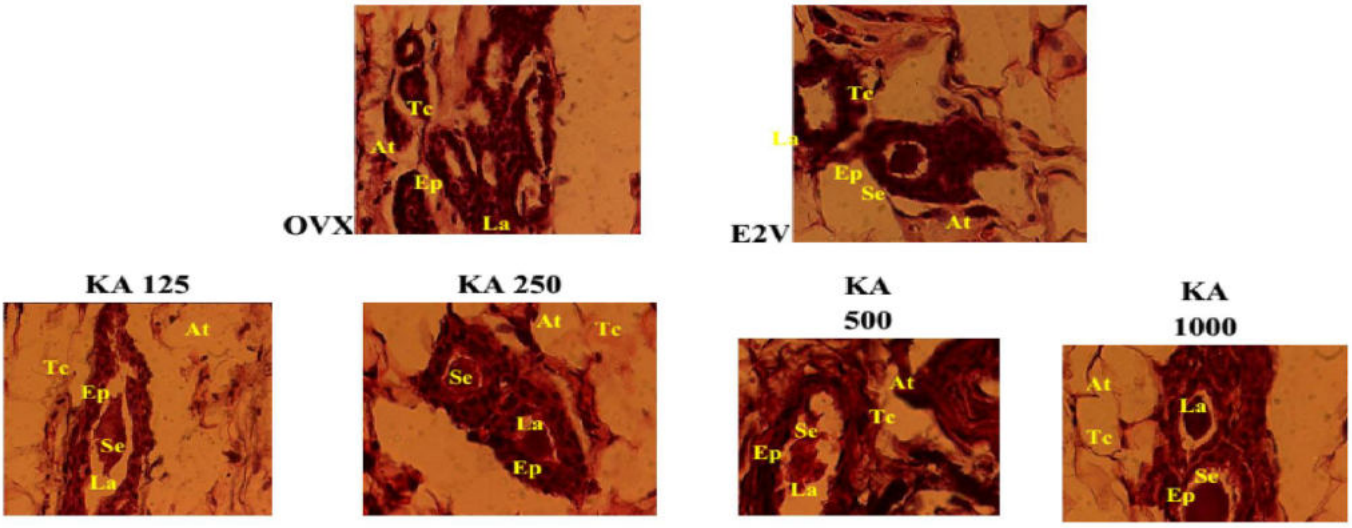

Figure3: effects of 3-day treatment with *khaya anthotheca* extract on mammary gland. N = 5 per group. Ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol valerate at 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. La = lumen of alveoli, ep = aveoli epithelium, at = adiposite tissue, tc = gland parenchyma, se = eosinophil secretion.

Results of the elevated plus-maze testwith *K. anthotheca* extract

Effect of *K. anthotheca* extract on percentage of time spent in open/closed arms of the elevated plus-maze

Compared which NOVX group, the ovariectomy induced a significant reduction of the percentage of time spent in the open arms ($p < 0.001$;passed from 7.68 ± 0.89% at NOVX group to 1.54 ±0.57% at OVX group). At the same time it induced a significant increase of the percentage of time spent in the closed arms ($p < 0.001$; passed from 92.32 ± 0.89% at NOVX group to 98.46 ± 0.57% at OVX group) (Figure. 4 A and B). The extract of *K. anthotheca* at all tested doses induced an increase in the percentage of time spent in the open arms (Figure. 4A) and a decrease of this percentage in the closed arms(Figure. 4B) compared with OVX group. Theses observed effects were significant at the dose of 1000 mg/kg BW ($p < 0.05$). Diazepam and E2V-treatment (1 mg/kg BW each) show the same effects. Compared with NOVX group, only the treatment with DZP induce a significant increase of the percentage of time spent in the open arms ($p < 0.05$) and a significant reduction of this percentage in the closed arms ($p < 0.05$) (Figure. 4 A and B).

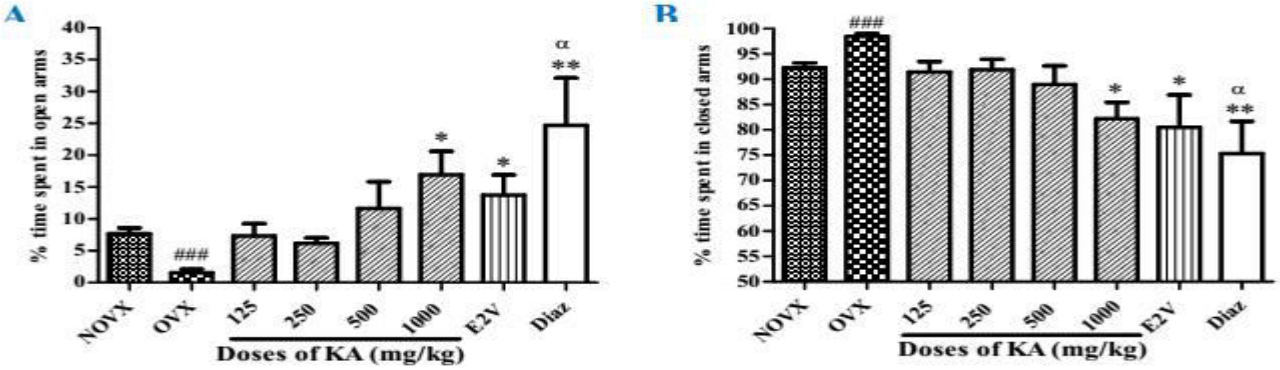

Figure4: effect of *khaya anthotheca* extract on % time spent in the open (a) and closed arms (b) of rats placed on the elevated plus-maze. Bars represent the percentage of time spent in the open and closed arms of the elevated plus-maze during 5 min. Data are expressed as mean ± sem, n = 8 per group. Novx = sham operated animals treated with the vehicle, ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol vale rate at 1 mg/kg bw, Diaz, diazepam, 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark extract of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. * $p< 0.05$ vs. Ovx; $p < 0.05$ vs. Novx (one-way anova followed by dunnett's test). ### $p< 0.001$ vs. Novx (unpaired t-test).

Effect of *K. anthotheca* extract on percentage of entries intoopen/closed arms of the elevated plus-maze

As show in Figure. 5 A and B, the ovariectomy induced a significant reduction of the percentage of number of entries into the open arms ($p < 0.05$) compared which NOVX group (passed from 27.14 ± 4.84% at NOVX group to 9.96 ± 3.32% at OVX group). At the same time it induced a significant increase of the percentage of number of entries into the closed arms ($p < 0.05$; passed from 72.86 ± 4.84% at NOVX group to 90.04 ± 3.32% at OVX group). The extract of *K. anthotheca* induced a significant increase in the percentage of number of entries into the open arms (Figure. 5A) and a significant decrease of this percentage in the closed arms (Figure. 5B) at all tested doses ($p < 0.05$; $p < 0.01$) compared with OVX group. Diazepam and E2V-treatment (1 mg/kg BW each) show the same effects (Figure. 5 A and B).

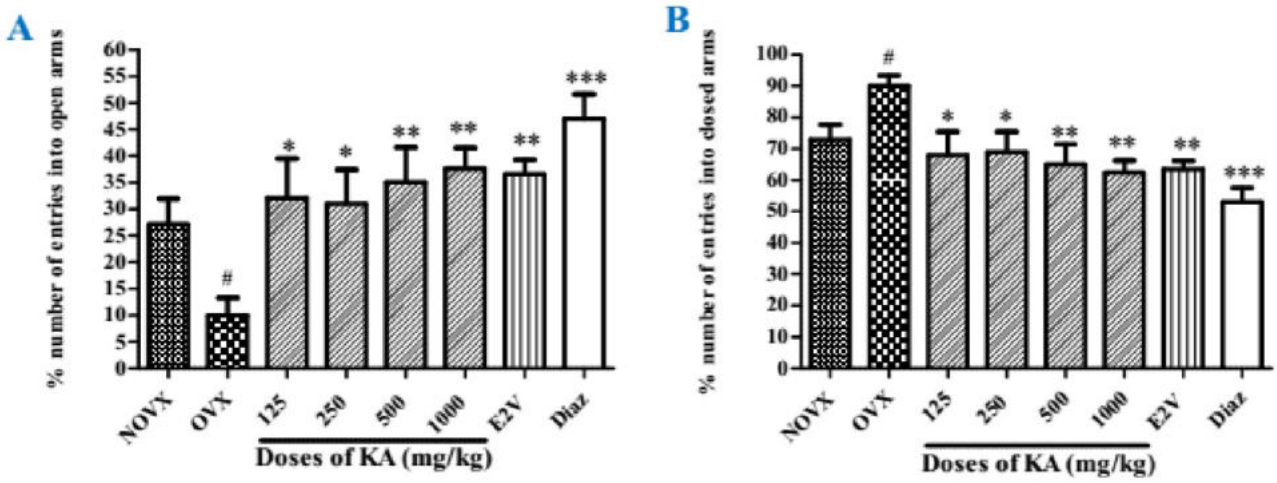

Figure 5: effect of *khaya anthotheca* extract on % number of entries into the open (a) and closed arms (b) of rats placed on the elevated plus-maze. Bars represent the percentage of number of entries into the open and closed arms of the elevated plus-maze during 5 min. Data are expressed as mean ± sem, n = 8 per group. Novx = sham operated animals treated with the vehicle, ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol vale rate at 1 mg/kg bw, diaz, diazepam, 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark extract of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. * p< 0.05, **p < 0.01, ***p < 0.001 vs. Ovx (one-way anova followed by dunnett's test). # p< 0.05 vs. Novx (unpaired t-test).

Effect of *K. anthotheca* extract on total arms entries, rearing, defecation and grooming

Analysis of the results depicted in Figure. 6A show that compared to NOVX group, the ovariectomy induced a non-significant reduction of the total arms entries in the arms of the labyrinth. The treatment witch *K. anthotheca* extract induced an increase of this total arms entries at all tested doses. Compared to OVX group (2.14 ±0.32),the total arms entries raised to 5.00 ± 0.70, 5.00 ±0.68 and 5.88 ±0.77 respectively for the doses of 250, 500 ($p < 0.05$) and 1000 mg/kg BW ($p < 0.01$). The same increase was observed with diazepam ($p < 0.01$) and E2V treatment at the dose of 1 mg/Kg BW each.

Compared to NOVX group, the ovariectomy induced a significant decrease of the number of rearing ($p < 0,001$) in the closed arms of the labyrinth (Figure. 6B). It rose from 9.31 ±0.24 in NOVX group to 5.67 ± 0.70 in OVX group. The treatment witch *K. anthotheca* extract induced an increase of this number of rearing at all tested doses compared to OVX group. This increase was significant at the dose of 1000 mg/kg BW ($p < 0.05$).The same increase was observed witch the diazepam ($p < 0.001$) and E2V treatments at the dose of 1 mg/Kg BW each.

As show in Figure.6C, the ovariectomy induced a significant increase ofthe number of defecation ($p < 0,001$) compared to NOVX group. The treatment with *K. anthotheca* extract at all tested doses induced a significant reduction of the number of defecation which passed from 3.20± 0.22 at OVX group to 1.29 ± 0.65, 1.29 ± 0.45, 1.38 ± 0.40 respectively for the doses of 125, 250 and 500 mg/kg BW ($p < 0.05$) and 0.83 ± 0.40 for the dose of 1000 mg/kg BW ($p < 0.01$). The diazepam and E2V treatments (1 mg/Kg PC each) induced each a significant decrease of the number of defecation ($p < 0.001$; $p < 0.01$ respectively) compared to OVX group.

Analysis of the results presented at the Figure. 6D show that the ovariectomy induced a significant increase of the number of grooming ($p < 0.05$) in the labyrinth compared to NOVX group. Treated rats witch *K. anthotheca* extract show a significant reduction of the number of grooming at the dose of 125, 250, 500 ($p < 0.05$) and 1000 mg/kg BW ($p < 0.01$). The same effects were

observed witch the diazepam and E2V treatments at the dose of 1 mg/Kg BW each ($p < 0.05$; $p < 0.001$ respectively).

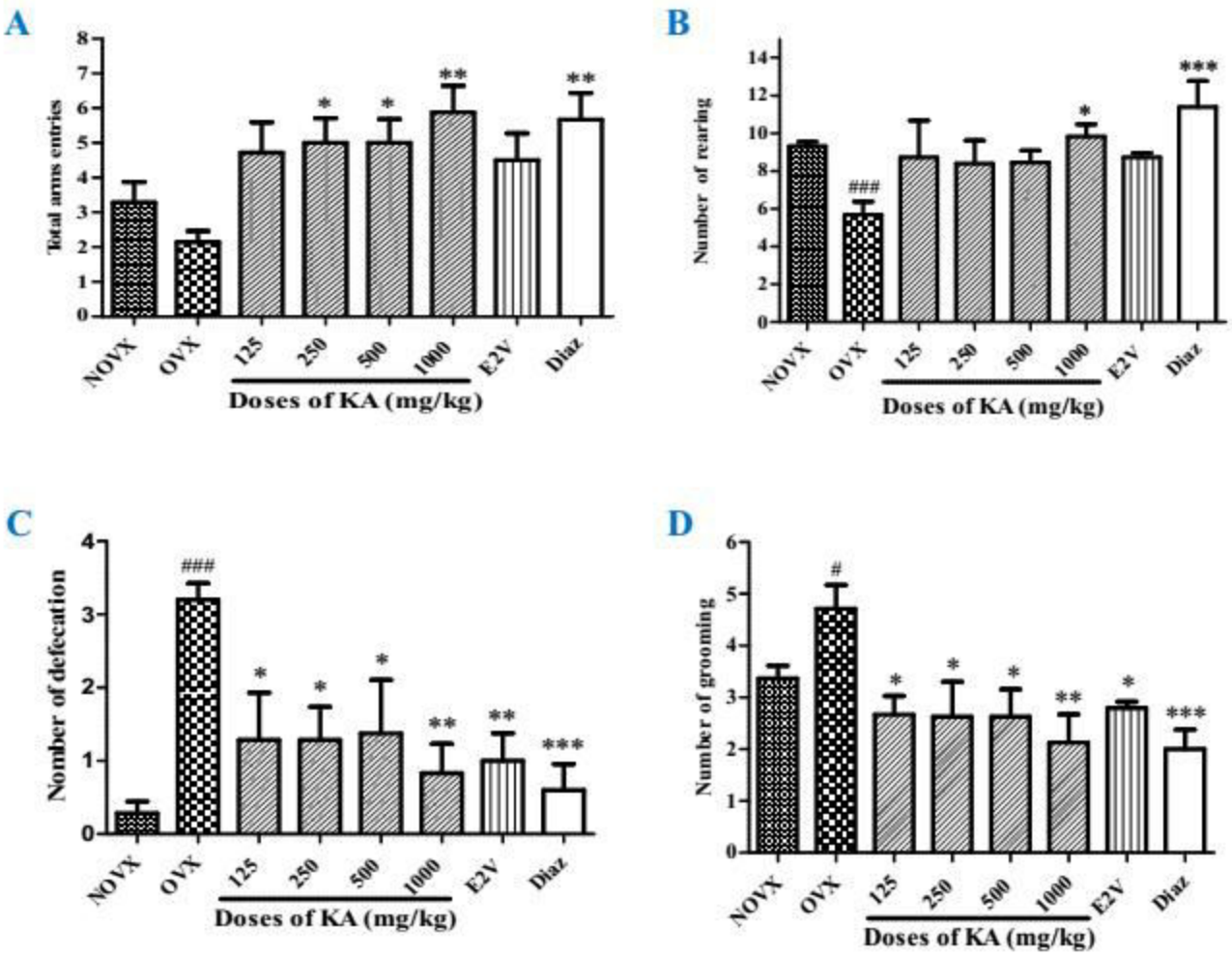

Figure 6: effect of *khaya anthotheca* extract on total arms entries, rearing, defecation and grooming of rats placed on the elevated plus-maze. Bars represent the total arms entries (a), number of rearing (b), number of defecation (c) and number of grooming (d)in the elevated plus-maze during 5 min. Data are expressed as mean ± sem, n = 8 per group. Novx = sham operated animals treated with the vehicle, ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol valerate at 1 mg/kg bw, diaz, diazepam, 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark extract of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. Ovx (one-way anova followed by dunnett's test). # $p < 0.05$, ### $p < 0.001$ vs. Novx (unpaired t-test).

Effect of *K. anthotheca* extract on rectal temperature of rats subjected to the elevated plus-maze test

Compared to NOVX group, the ovariectomy induced a significant increase of the rectal temperature in rats ($p < 0.01$). It ranged from 35.75 ± 0.14 at NOVX to 36.34 ± 0.09 at OVX group(Figure. 7). The rats treated with the aqueous extract of *K. anthotheca* (at all tested doses) show a reduction of the rectal temperature. Figure 7 also shows that compared to OVX group, this decrease was significant at the doses of 125, 250 and 500 mg/kg BW ($p < 0.05$). Treatment with E2V induced the same effects ($p < 0.05$).

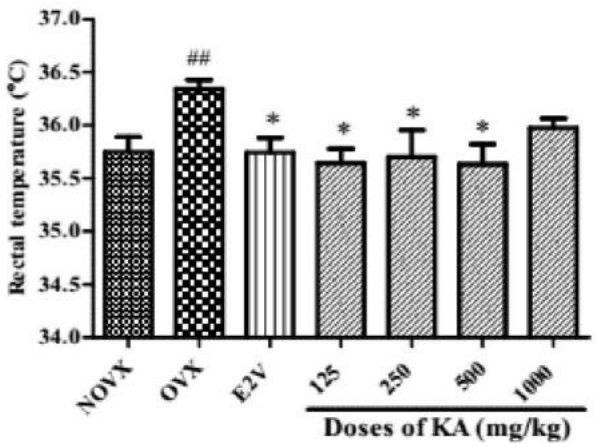

Figure 7: effect of *khayaanthotheca* extract on rectal temperature of rats submits to the elevated plus-maze test. Bars represent rectal temperature. Data are expressed as mean ± sem, n = 8 per group. Novx = sham operated animals treated with the vehicle, ovx = ovx animals treated with the vehicle, e2v = ovx animals treated with estradiol valerate at 1 mg/kg bw, diaz, diazepam, 1 mg/kg bw, ka = ovx animals treated with the aqueous extract of stem bark extract of *khaya anthotheca* at the doses of 125, 250, 500 and 1000 mg/kg bw. * p< 0.05 vs. Ovx (one-way anova followed by dunnett's test). ## p< 0.01 vs. Novx (unpaired t-test).

Discussion

To evaluate the estrogenic effects of *Khaya anthotheca* extract, a 3-day uterotrophic assay in ovariectomised adult female rats was carried out. This ovariectomy model also considered as postmenopausal-like model is usually used by different laboratories to investigate the effects of natural and synthetic products on menopausal symptoms [24, 25, 33, 34, 35].

As expected estrogen deficiency was accompanied with an atrophy of the uterus in OVX animals. A 3-day treatment with E2V induced a significant increase in uterine wet weight and uterine epithelial thickness. The increase in the uterine weight is mainly attributed to the uterine water imbibition and/or cell proliferation [36] and these effects are known to be mediated through ER [25, 37]. The treatment with *K. anthotheca* extract did not induce any modification of these parameters. Regarding vaginal epithelial height, compared to the OVX group, a 3-days treatment with E2V and *K. anthotheca* extract at the doses of 250, 500 and 1000 mg/kg BW induced a significant increase. Furthermore, these observations are in agreement with several studies, which shown that estrogen and estrogenic compounds influence vaginal epithelium by inducing the cells proliferation and differentiation to give stratification and cornification of vagina [25, 38, 39]. This result suggests that *K. anthotheca* extracts could alleviate vaginal dryness experienced at menopause. The mammary gland was also studied in this work as an estrogen target organ. *K. anthotheca* extract at all tested doses increased the diameter and the lumen of alveoli, and an abundant eosinophil secretion in lumen of alveoli. These results are in accordance with the observations made by some authors [25, 38], in which estrogen-like substances reversed mammary gland regression induced by ovariectomy.

The literature revealed that estrogens play an important role in body temperature maintenance in women [40]. In our investigation, like 17β-estradiol, *K. anthotheca* extract induced a decrease of the rectal temperature measured in rats after de EPM test compared to OVX group. These results suggest that *K. anthotheca* extracts may contain estrogenic compounds endowed with estrogenic properties. The observed effects could justify the use of Khaya genus in the treatment of fever in African traditional medicine [17].

Taken alltogether, the estrogenic property of *K. anthotheca* extract may be tissues specific. This specificity could be due to the binding of estrogenic compounds present in the *K. anthotheca* extract on different types of estrogens receptors. It is known that the uterine have the two types of estrogens receptors (and β) and it was reported that ERβ mediated anti-proliferative effects or antagonized the actions of ER [41]. This suggest that in the uterine, the secondary metabolites present in the aqueous extract of *K. anthotheca* would induce selective effects while acting like agonist of the type of ERβ and as antagonist of the type of ER on the same organ. The estrogenic specificity of extracts on the estrogen target organs can be verified by histological examination of the uterus, the vagina and the mammary glands [25, 42].

According to the World Health Organization [3], anxiety is the most common psychiatric disorder and it significantly affects quality of life and familial, social, and economic environments of the world population [43]. So that an interesting source of new anxiolytic substances comprises medicinal plants, whose metabolic diversity leads to study in search of important therapeutic agents [44].To achieve this objective, the elevated plus-maze (EPM) test was used. It is the most commonly test used to study anxiolytic response of almost all types of anti-anxiety agents. The EPM test has many merits: the test is fast and simple; it is based on spontaneous behavior; it is able to identify acute anxiolytic effects of drugs and it is bi-directionally sensitive to manipulations of anxiety. Given this profile, this test is used in routine anxiolytic drugs screening and in the study of the mechanisms of anxiety [45]. The results obtained in our investigation with the EPM test showed that ovariectomy induce an increase of the percentage of closed arms entries and time spent in the same arms. In the open arms, a decrease of the same parameters was observed. All these effects reflect a decrease of open arms exploration and showed the anxiogenic responses of ovariectomyin the EPM. These anxiogenic effects of ovariectomy are also materialized by an increase of defecation and grooming and a decrease of total arms entries and rearing [35]. The rats receiving the aqueous extract of *K. anthotheca* showed an increase in the percentage of entries into, and time spent in open arms, when compared with those of the OVX group; as well as a reduced percentage of entries into, and time spent in closed arms. According to Thakur and Mengi [46],

Oviedo et *al.* [47] and Ketcha *et al.* [32], the increase in the activity in the open arms directly reflects a reduction of the anxiety and the reduction in the activity in the closed arms shows a decrease of the stress. These effects observed at the same time in the open arms and closed arms suggest that the aqueous extract of *K. anthotheca* may contain compounds endowed with anxiolytic properties like suggested by Grundmann et *al.* [48] and Ngo Bum et *al.* [30]. The treatment with *K. anthotheca* extract also induced an increase of total arms entries and rearing. Moreover, there was a decrease of defecation and grooming at all tested doses and these effects reflects an increase of locomotors activity. Some authors show that extracts that acting like diazepam and 17β-estradiol by increasing the open arm exploration in the EPM, without change the total arms entries, an index of locomotor activity have anxiolytic activity [28, 49, 50].

Data obtained from our study indicate the anxiolytic action of *K. anthotheca* and they are in accordance with earlier reports on anxiolytic action of *K. anthotheca*. However, this is the first study in our knowledge demonstrating the anxiolytic effects of this drug in EPM paradigm in rats. Our finding that *K. anthotheca* treatment ameliorates ovariectomy-induced anxiety is in accordance with a report that drugs of natural origin can be useful in stress-induced anxiety [51]. Anxiolytic drug are one class of compounds intended to fight the psychic and somatic components of anxiety. Several classes of compounds showed their anxiolytic efficacy by activation of receptors GABA and/or ER β on the brain [35, 52, 53]. These anxiolytics properties of *K. anthotheca* extract could result from the action of some compounds funds in Khaya genus like saponins on gamma amino-butyric acid (GABA) receptors complex [52]. The 3-day uterotrophic assay showed that the *K. anthotheca* extract has and estrogenic activity so that it could contain estrogens compounds (phytoestrogens) and this action may be tissues specific. These phytoestrogens like flavonoids found in this genus, that present a structural and functional similarity with estrogens can activate GABAA receptors on his steroids site fixation [34] and the ER β that play a major role in the regulation of the anxiety in the brain[35, 53].

Conclusion

The aim of this study was to evaluate the estrogenic and anxiolytic effects of the decoction of *K. anthotheca* in ovariectomised rats. Our findings have shown that *K. anthotheca* extract induced a significant estrogen-like activity on some estrogen target organs (vagina and mammary gland) and induced a reduction of therectal temperature. In addition the extract also exhibits anxiolytic properties in ovariectomised rats. These properties could justify the traditional use of this plant in African traditional medicine to manage fever, anxiety and to alleviate vaginal dryness in postmenopausal women.

Conflict of interest

All the authors state that there are no conflicts of interest within this article.

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