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Anxiolytic, estrogenic and non-toxic effects of sub-acute treatment with *Khaya anthotheca* (Welw.) C.DC (Meliaceae) decoction in ovariectomized Wistar rats

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ABSTRACT

In Cameroon, Khaya anthotheca (Welw.) C.DC (Meliaceae) is traditionally used to manage central nervous system diseases. Then, the purpose of this study was to evaluate the anxiolytic, estrogenic and toxic effects of sub-chronic treatment with Khaya anthotheca (K. Anthotheca) extract using ovariectomized Wistar rats. For this to be done, 36 rats were ovariectomized and the 6 others were used as SHAM rats. 14 days after ovariectomy, animals were distributed into six groups of 6 animal each. They were treated for 28 consecutive days with distilled water, K. Anthotheca extract (at the doses of 125, 250, and 500 mg/kg BW), estradiol valerate or diazepam. The anxiolytic activity was investigated through the elevated plus-maze (EPM) test; the estrogen-like effects of the extract were determinate on primary estrogens targets and his toxicity was evaluate. As results, in the EPM test, sub-acute treatment with K. Anthotheca extract increased rearing ($p < 0.01$), decrease the weight of fecal boli ($p < 0.01$; $p < 0.05$) and number of grooming ($p < 0.01$). The extract increased ($p < 0.01$; $p < 0.001$) the vaginal epithelia height and stimulated the eosinophil secretion on lumen of alveoli of the mammary gland. In the acute toxicity and sub-acute treatment, the extract showed neither mortality nor exterior toxicity signs ($LD_{50} > 5000mg/kg$). The sub-acute treatment with the extract at the doses of 125, 250 and 500 mg/kg BW did not present any histological modification of liver, kidneys and lungs. These results suggest that K. anthotheca is endowed with anxiolytic and estrogenic properties. Moreover, these results indicate a broad safety margin for K. anthotheca decoction.

Keywords: *Khaya anthotheca*, ovariectomy, estrogenic properties, anxiolytic effects, toxicological profile.

INTRODUCTION

Menopause, which occurs around 50 ± 5 years old, is characterized by the permanent cessation of menses and linked to the loss of ovarian activity. It was reported that estrogen depletion is associated with cognitive disfunction, depression and anxiety (Walf and Frye, 2007). Nowadays, menopausal women look alternative treatment to modern medicine by using natural health drug (Pitkin, 2012). It is well known that medicinal plant provided wide variety of natural drug (Wong, *et al*., 2006) and in most developing countries, for their primary health care, the majority (80%) of the population use traditional medicine (WHO, 2008). The search for alternative medication, even in developing countries, by the use of plant extracts, increased in the menopausal and postmenopausal population (Low Dog, 2005; Scheid, 2007). However, because they are "natural", herbal drugs are considered to be safe and free from side effects. Base on this, general public is encouraging to use extracts plant for self-medication. Thus, it is important to carried out pharmacological and toxicological studies to validate the usage of traditional preparations (David *et al.,*2015).

Khaya anthotheca is an african medicinal plant used to treat diseases like helmenthiasis, malaria, gonorrhea and abdominal pain (Toyang *et al*., 1995; Amri and Kisangau, 2012). Khaya genus plant is used to manage convulsion, fever, stomach ache and rheumatism (Ojokuku *et al*., 2010). The phytochemical characterization of *Khaya* genus revealed the presence different class of compounds such as polyphenols (flavonoids) and saponins (Ojokuku *et al*., 2010; Petruczynik, 2012; Njayou*et al*., 2015). In recent studies, we have demonstrated the anxiolytic and estrogenic effects of the decoction of this plant in acute treatment (Ketcha *et al*., 2017), his ameliorative effects on neurotoxicity induce by vanadium (Zemo *et al*., 2021a) as well as his neuroprotective effects in ovariectomy-induced neurodegeneration model (Zemo *et al*., 2021b). To manage physiological dysfunctions due to menopause, women have to use the treatment for many days and for these mentioned reasons, the purpose of this study was to evaluate the anxiolytic, estrogenic and toxic effects of sub-acute treatment with decoction of *Khaya anthotheca* on ovariectomized Wistar rats.

MATERIAL AND METHODS

Animals

Animals were handled according to the European Community Guidelines for Laboratory Animal Use and Care (EEC Directive of 1986; 86/609/EEC). Juvenile female Wistar rats (120 - 160 g body weight, 10 - 12 weeks old) were used for this study. Animals were housed at the Laboratory of Physiology of the University of Yaounde I (t°: 25° C; humidity: 50-80%; 12 h light-dark cycle). They had free access to standard soy-free rat diet, except in the acute toxicity study. Animals were provided tap water ad libitum.

Plant material and extraction

The stem bark of *Khaya anthotheca* were collected in Mamougnam (District of Massangam, Department of Noun, West Region of Cameroon). The plant was identified by comparison to the specimen number 4230/HNC at the National Herbarum of Cameroon (HNC). The decoction was obtained as previously describe (Ketcha *et al.,* 2017).

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

For the sub-acute treatment, 42 female Wistar rats were used. Thirty six (36) rats were ovariectomized (OVX) using the dorsal approach (Lane *et al*., 2003) under diazepam /ketamine anesthesia (respectively 10mg/kg and 50mg/kg BW; i.p.) and the 6 remaining rats were used as SHAM rats. Fourteen days after endogenous hormonal decline (OECD, 2007), animals were randomly distributed in seven groups (n = 6):

Group 1: SHAM = Sham operated, rats treated with the vehicle (distilled water, p.o.);

Group 2: OVX = ovariectomized rats treated with the vehicle (p.o.);

Group 3: KA 125 = OVX rats treated with the aqueous extract of *Khaya anthotheca* at 125 mg/kg BW (p.o.);

Group 4: KA 250 = OVX rats treated with the aqueous extract of *Khaya anthotheca* at 250 mg/kg BW (p.o.);

Group 5: KA 500 = OVX rats treated with the aqueous extract of *Khaya anthotheca* at 500 mg/kg BW (p.o.);

Group 6: E2V = OVX rats treated with estradiol valerate at 1 mg/kg BW (p.o.);

Group 7: Diaz = OVX rats treated with diazepam at the dose of 1 mg/kg BW (i.p.).

Body weights were measured before the beginning of treatments and then weekly until the end of the study. At the 28th treatment day, animals were submitted to the elevated plus-maze (EPM) test one hour after administration of different substances. Rectal temperature of each rat was taken at the end of EPM test. Animals were sacrificed under anesthesia by using diazepam (10 mg/kg BW) and ketamine (50 mg/kg). Organs such as liver, kidneys, lung, spleen, adrenals, stomach, right femur and uterine were excised and weighted. Uterine, vagina, mammary gland, liver, kidney and lung were fixed in 10% formaldehyde for histological analysis.

Elevated plus-maze test

Elevated plus-maze is the most and simplest apparatus used for the screening of anti-anxiety drug. The elevated plus -maze 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 animal present in the EPM has a preference to explore the enclose arms. Anxiety is marked by immobility and defecation. This study was carried out according to the protocol describe by Ketcha *et al.* (2017).

The parameters noted were time spent in open/closed arms, number of entries in the open/closed arms, number of rearing, weight of fecal boli and number of grooming (Casarrubea *et al*., 2012; Ketcha *et al*., 2015). The percentages of times spent and the number of entries in each type of arms were calculated for each animal. After the test, the rectal temperature of each animal was measured using a medical thermometer.

Acute oral toxicity

The guideline Nr.423 (OCDE, 2001a) of the acute toxicity class method provide by the Organization of Economic Cooperation and Development (OCDE) was used with slight modification (Awounfack *et al*., 2016). For this experiment, female rats were used according to literature surveys of

conventional LD_{50} tests which reported that females are generally slightly more sensitive (Lipnick *et al.,* 1995) and OECD recommendation to use female animals in acute toxicity. Animals were randomly distributed into two groups (n = 3):

Group 1: Control = normal animals treated with the vehicle (distilled water);

Group 2: KA 5000 = normal animals treated with the *Khaya anthotheca* decoction freshly prepared at 5000 mg/kg BW.

All animals were treated by gavage with an administration volume of 2 mL/100g BW. The experiment was carried out in two time using different animals. Signs of toxicity were base on mortality, respiratory pattern, change in general behavior, skin, eyes fur and somatomotor activity. The animals were weighed before treatment and every 3 days during the post-treatment period of 14 days. Fourteen days after, all animals were weighed and sacrificed under anesthesia using diazepam (10 mg/kg BW) and ketamine (50 mg/kg BW). Organs including liver, kidneys, lung, spleen, adrenals, stomachs, heart, ovaries, uterine, brain and right femur were excised and weighed.

Histological analysis

Microarchitecture of mammary glands, uterine, vagina, liver, kidneys and lung were assessed from 5-µm section of paraffine-embedded tissues following hematoxylin-eosin staining. All organs were photographed using the complete Zeiss equipment as describe by Njamen *et al*., 2007 and Ketcha *et al* 2017.

Statistical analysis

Results were expressed as mean ± S.E.M. The one-way ANOVA followed by Dunnett's test was used to compare treated groups and OVX group or SHAM group and the unpaired t-test was used to compare OVX group and SHAM (sub-acute treatment). In acute toxicity study, the difference between Control group and KA 5000 group was determined using the two-way non-parametric Mann & Whitney U test (GraphPad Prism, version 5.03). A p-value < 0.05 was considered significant.

RESULT AND DISCUSSION

Anxiolytic effects of sub-acute treatment with decoction of *Khaya anthotheca* evaluated by elevated plus-maze test

- **Effect of *Khaya anthotheca* extract on percentage of time spent in open/close arms of the elevated plus-maze**

In OVX group, the percentage of time spent in open arms (Fig. 1A) decreased ($p < 0.001$) while the percentage of time spent in the close arms (Fig. 1B) increased ($p < 0.001$) compared to the age-matched SHAM group. *Khaya anthotheca* extract induced a non-significant variation of these parameters. Compared to OVX (1.25 ± 0.28%), the dose of 250 mg/kg BW increase the percentage of time spent in the open arms (7.84 ± 2.97%) (Fig. 1 A). The same dose also induced a decrease of this percentage in the closed arms (passed from 98.75 ± 0.28% at OVX group to 92.16 ± 2.97% at KA 250 group) in comparison to OVX group (Fig. 1 B). Compared to OVX group, only the treatment with Diazepam, use as reference drug, induce a significant increase of the percentage of time spent in the open arms ($p < 0.05$) and a significant reduction of the percentage of time spent in the close arms ($p < 0.05$) (Fig. 1 A and B).

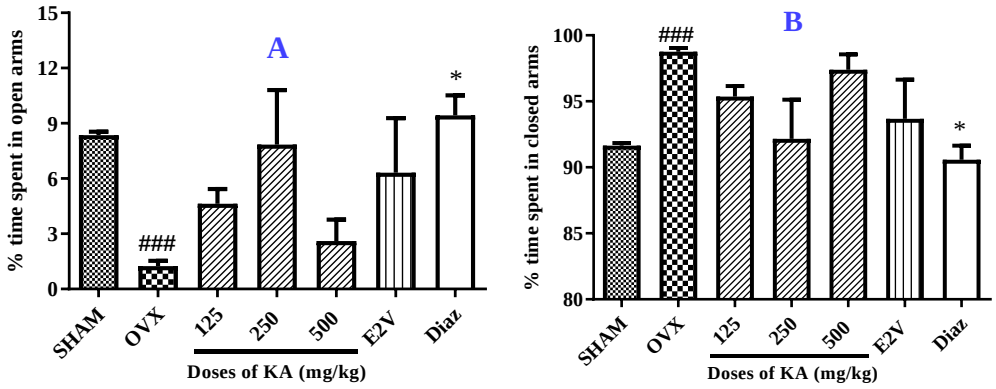


Figure 1: Effect of *Khaya anthotheca* extract on percentage of time spent in open (A) and close (B) arms of rats placed on the elevated plus-maze.

Bars represent the % of time spent in the open and close arms of the EPM during 5 min. Data are expressed as mean ± SEM, n = 6. * $p < 0.05$ vs OVX (one-way ANOVA followed by Dunnett's test). ### $p < 0.001$ vs. SHAM (unpaired t-test).

- **Effect of *Khaya anthotheca* extract on percentage of entries into open/closed arms of the elevated plus-maze**

Analysis of figure 2 revealed that ovariectomy induced a significant ($p < 0.01$) decrease of the percentage of the percentage of the number of entries into the open arms (Fig. 2A) and a significant ($p < 0.01$) increase of the percentage of number of entries into the closed arms (Fig. 2B). The extract of *K. anthotheca* induced a non-significant increase in the percentage of number of entries into the open arms (Fig.2A). Compared to OVX (16.67 ± 4.30%), the dose of 250 mg/kg BW increase the percentage of number of entries into the open arms (26.67 ± 9.23%) (Fig. 2 A). The same dose also induced a decrease of this percentage in the closed arms (passed from 83.33 ± 4.30% at OVX group to 73.33 ± 9.3% at KA 250 group) in comparison to OVX group (Fig. 2 B). Only the treatment with diazepam, use as reference drug, induce a significant increase of the percentage of number of entries into the open arms ($p < 0.05$) and a significant reduction of this percentage in the closed arms ($p < 0.05$) compared to OVX group (Fig. 2 A and B).

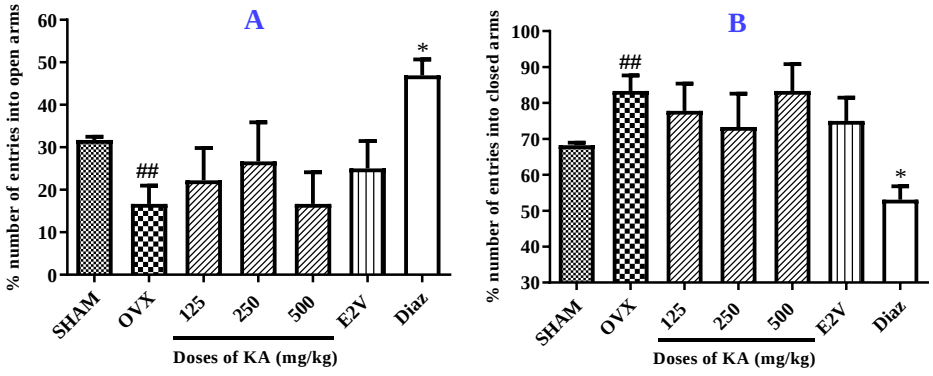


Figure 2: Effect of *Khaya anthotheca* extract on percentage of number of entries into the open (A) and close (B) arms of rats placed on the elevated plus-maze.

Bars represent the % of number of entries into the open and close arms of the EPM during 5 min. Data are expressed as mean ± SEM, n = 6. * $p < 0.05$ vs OVX (one-way ANOVA followed by Dunnett's test). ## $p < 0.01$ vs. SHAM (unpaired t-test).

- **Effect of Khaya anthotheca extract on rearing, weight of fecal boli and grooming**

Compared to SHAM group, ovariectomy induced a significant ($p < 0.001$) decrease of rearing in the closed arms of the labyrinth (Fig. 3A). At all tested doses, administration of *K. anthotheca* extract induced an increase of rearing compared to OVX group. This increase was significant at the dose of 250 mg/kg BW ($p < 0.01$). The same increase ($p < 0.001$) was observed with the diazepam and E2V treatments at the dose of 1 mg/Kg BW each in comparison to OVX group.

As shown in figure 3 B, OVX animals showed an increase of the weight of fecal boli ($p < 0.01$). Administration of *K. anthotheca* at all tested doses, induced a significant ($p < 0.001$; $p < 0.01$ and $p < 0.01$) reduction of this parameter. The diazepam and E2V treatments (1 mg/kg BW each) induced a significant ($p < 0.001$) decrease on the number of defecation in comparison to OVX group.

Analysis of the number of grooming produced in the labyrinth show no significant variation between SHAM and OVX group (Fig. 3 C). Rats treated with *K. anthotheca* extract at the 500 mg/kg BW show a significant ($p < 0.01$) reduction of the number of grooming. The same effects were observed with diazepam and E2V treatments at 1 mg/Kg BW each ($p < 0.01$) in comparison to OVX group.

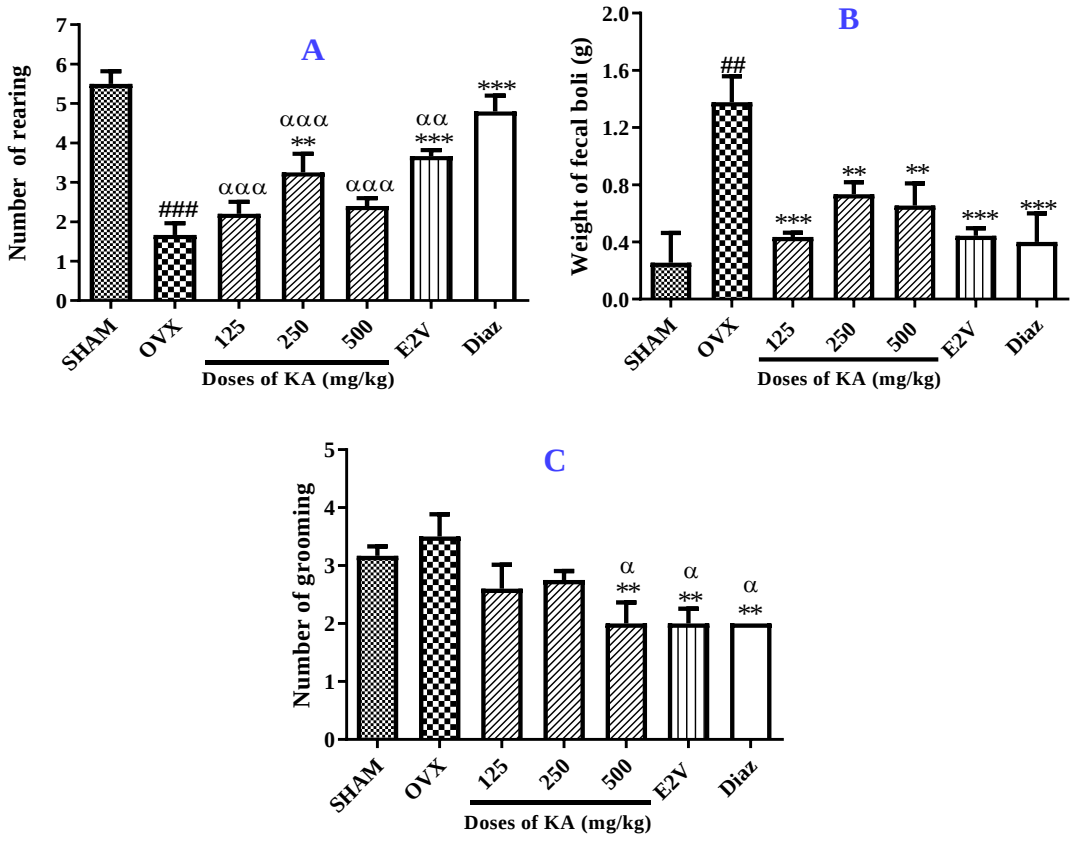


Figure 3: Effect of *Khaya anthotheca* extract on rearing, weight of fecal boli and grooming of rats placed in the elevated plus-maze.

Bars represent the number of rearing (A), weight of fecal boli (B) and number of grooming (C) in the EPM during 5 min. Data are expressed as mean ± SEM, n = 6. ** $p < 0.01$, *** $p < 0.001$ vs OVX; α $p < 0.05$, αα $p < 0.01$, ααα $p < 0.001$ vs SHAM (one-way ANOVA followed by Dunnett's test). ## $p < 0.01$, ### $p < 0.01$ vs. SHAM (unpaired t-test).

- **Effect of *Khaya anthotheca* extract on rectal temperature of rats submitted to the elevated plus-maze test**

Analysis of the results presented in figure 4 show that compared to SHAM group, ovariectomy induced a significant ($p < 0.05$) increase of the rectal temperature in rat. The rats treated with the aqueous extract of *K. anthotheca* (at all tested doses) show a reduction of the rectal temperature compared to OVX group, this decrease was significant ($p < 0.05$) at 500 mg/kg BW. Treatment with E2V induced the same effects ($p < 0.05$) as the extract in comparison to OVX animals.

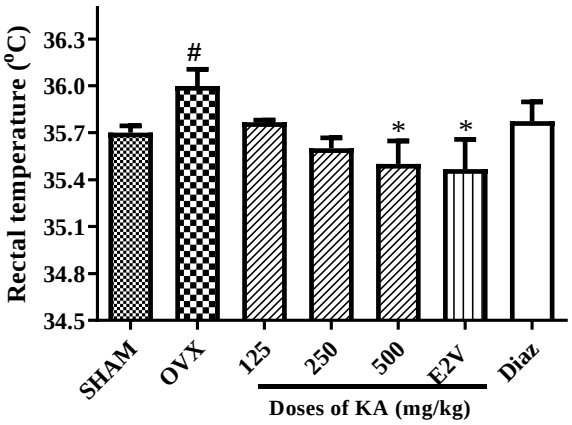


Figure 4: Effect of *Khaya anthotheca* extract on rectal temperature of rats submitted to the elevated plus-maze test.

Bars represent rectal temperature. Data are expressed as mean ± SEM, n = 6. * $p < 0.05$ vs OVX (one-way ANOVA followed by Dunnett's test). # $p < 0.05$ vs. SHAM (unpaired t-test).

Estrogenic effects of sub-acute treatment with decoction of *Khaya anthotheca* on primary estrogens targets

The results obtained after 28-days of treatment showed that ovariectomy induced a significant ($p < 0.01$) decrease of the uterine wet weight (Fig. 5A), uterine and vagina epithelial thickness (Fig. 5 B, 5C and 6), and inhibit the eosinophilic secretion on the mammary gland (Fig. 7) compared to the SHAM group. As shown in Figure 5, 6 and 7, E2V at 1mg/kg BW has corrected ($p < 0.001$) all those effects induced by ovariectomy. *K. anthotheca* extract has the same significant ($p < 0.01$; $p < 0.001$) effects on vagina (Fig. 6) and mammary gland (Fig. 7). There was a non-significant effect induce by the extract on uterine epithelial height.

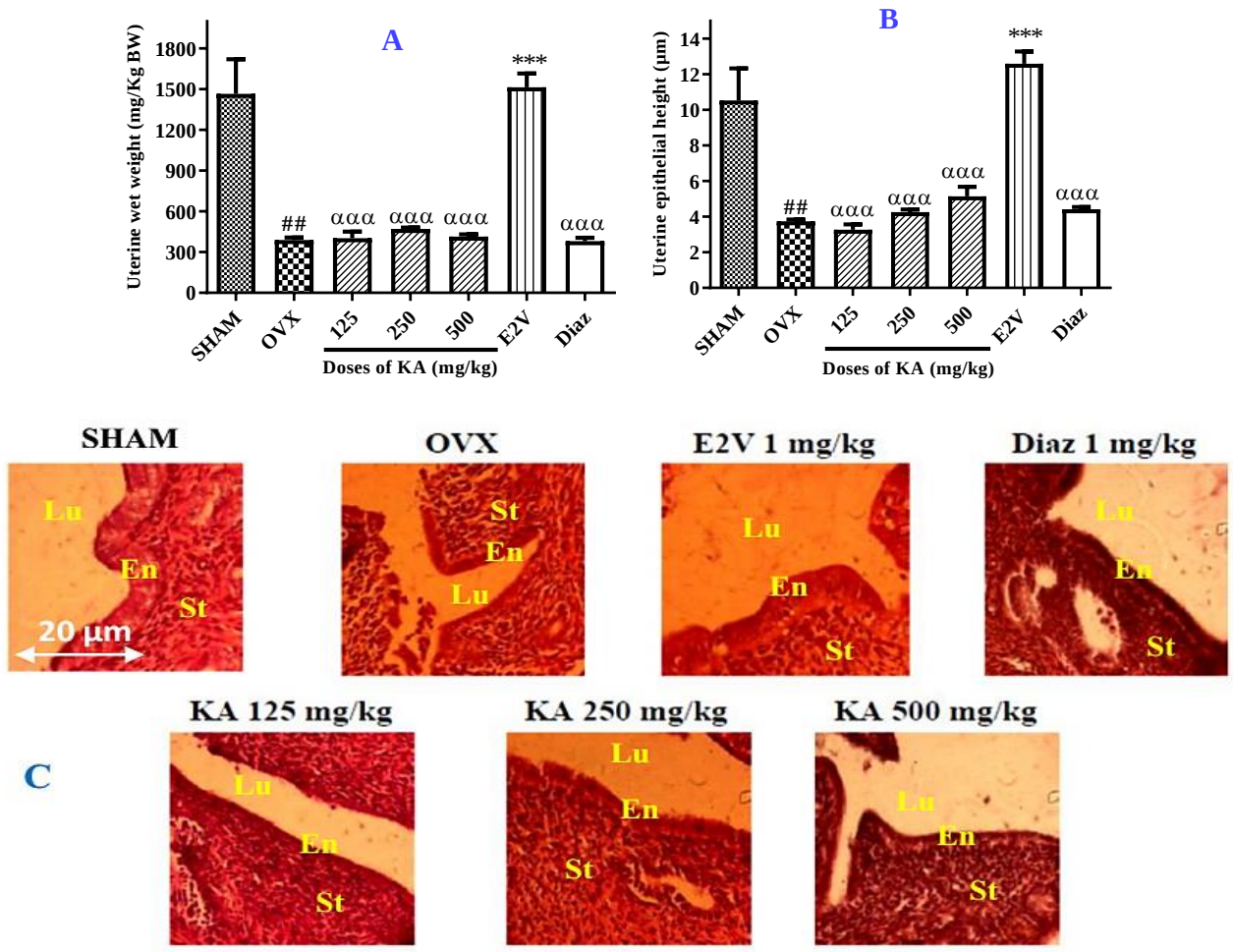
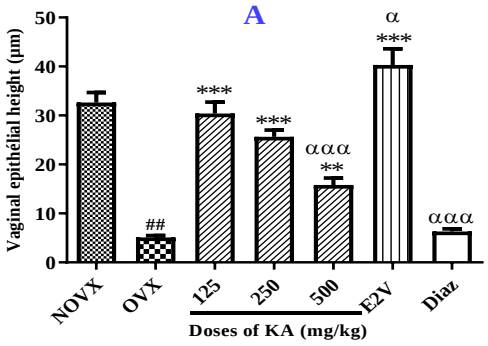


Figure 5: Effects of 28-days treatment with *Khaya anthotheca* extract on the uterine wet weight and uterine epithelial thickness.

Bars represent the uterine wet weight (A) and uterine epithelial thickness (B). Data are expressed as mean ± SEM, n = 6; microphotographs (C). ***$P < 0.001$ vs. OVX; ααα $p < 0.001$ vs. NOVX (one-way ANOVA followed by Dunnett's test), ##$P < 0.01$ vs. SHAM (Mann-Whitney test). Lu: uterine lumen; En: Endometrium; St: Stroma.



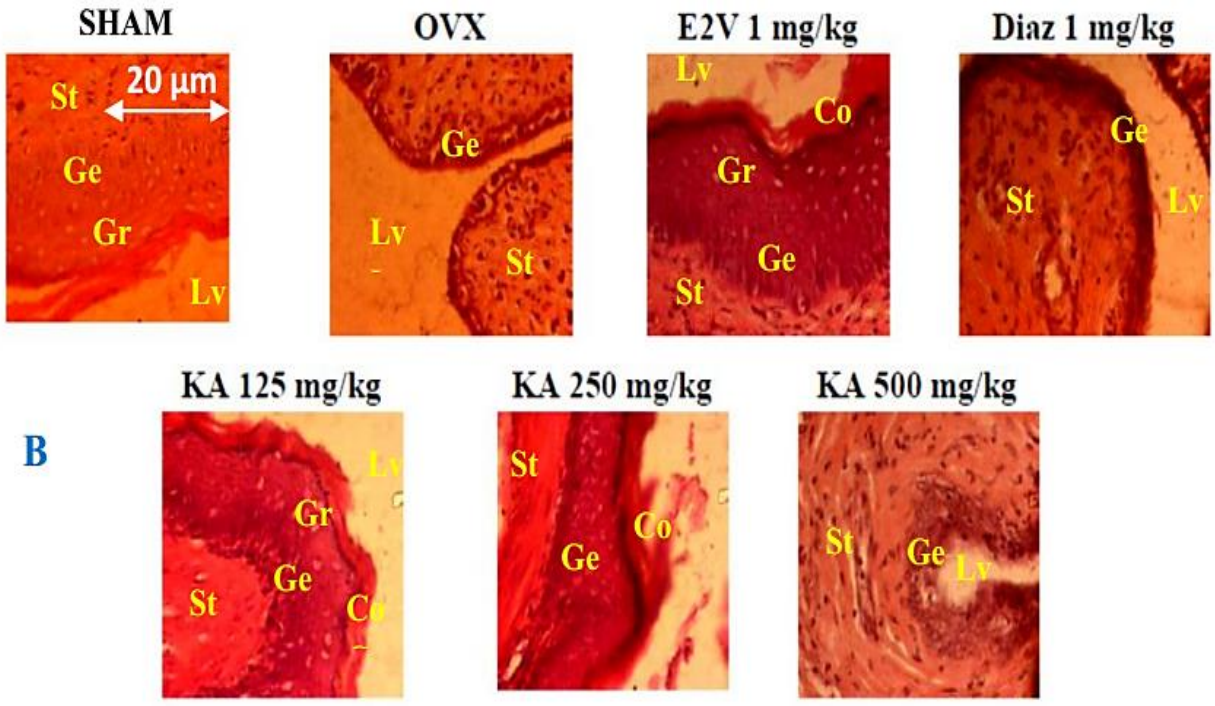


Figure 6: Effects of 28-day treatment with *Khaya anthotheca*extract on the vaginal epithelial thickness.

Bars represent the epithelial height (A). Data are expressed as mean ± SEM, n = 6; microphotographs (B). $^{**}P < 0.01$, $^{***}P < 0.001$ vs. OVX; α $p < 0.05$, ααα $p < 0.001$ vs. NOVX (one-way ANOVA followed by Dunnett's test), $\#\#P < 0.01$ vs. SHAM (Mann-Whitney test). Lv = vaginal lumen, Co = stratum corneum, Gr = stratum granulosum, Ge = stratum germinativum, St = Stroma.

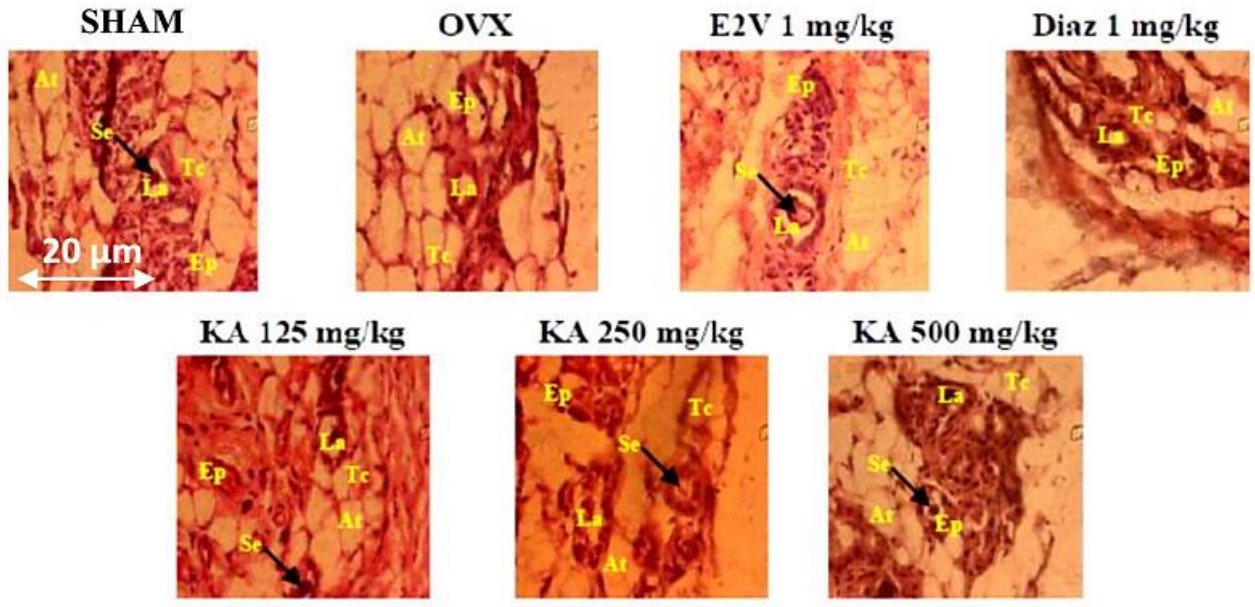


Figure 7: Effects of 28-days treatment with *Khaya anthotheca*extract on mammary gland.

Microphotographs, n = 6. La = lumen of alveoli, Ep = aveoli epithelium, At = adiposite tissue, Tc = gland parenchyma, Se = eosinophil secretion.

Acute oral toxicological evaluation

No mortality or phenotypical signs of toxicity over the 14-days observation period was noted after the oral administration of a single dose (5000 mg/kg) of the *Khaya anthotheca* extract, even after the repetition of the experiment. This indicated that the LD_{50} value must higher than 5000 mg/kg. In the body weight profile (Fig. 8) and relative weight of selects organs (Table1), no significant differences were found in treated groups compared to controls. Similarly, no changes in the behavior, respiratory pattern, skin, eyes, fur and somatomotor activity were reported. No gross pathological signs an no significant differences were revealed by the necropsy analysis.

Table 1: Relative organ wet weights (mg/kg BW) of selected organs of rats following a single oral administration of KA at the dose of 5000 mg/kg BW.

Organs	Control	KA 5000
Liver	30653 ± 843	36246 ± 424
Kidneys	6852 ± 028	6897 ± 041
Lungs	9807 ± 224	6912 ±245
Spleen	4466 ± 193	5589 ± 201
Adrenals	506 ± 034	513 ± 014
Stomach	9812 ± 558	9169 ± 259
Heart	3438 ± 093	3656 ± 188
ovaries	889 ± 014	753 ± 014
Uterine	2822 ± 626	2728 ± 836
Brain	6790 ± 249	6905 ± 263
Right femur	2535 ± 047	2502 ± 058

Data are expressed as mean ± SEM, n = 3. No significant difference.

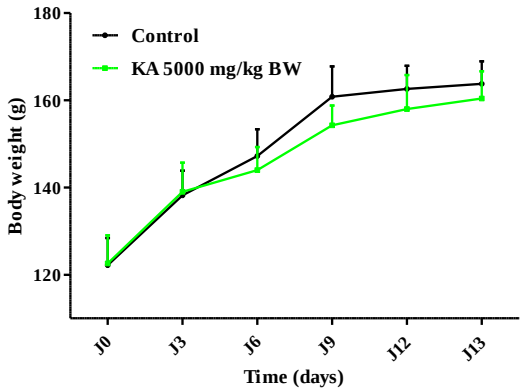


Figure 8: Body weight variation of rats after administration of a single dose of 5000 mg/kg BW of the *Khaya anthotheca* extract.

Each point represents the mean ± ESM (n = 6). No significant difference.

Toxicological profile of *Khaya anthotheca* decoction after a sub-chronic treatment

After 28 days of treatment with the aqueous extract of stem bark of *Khaya anthotheca* at the doses of 125, 250 and 500 mg/kg BW, the extract did not induce any significant variations in relative weights of liver, kidneys, lung, spleen, adrenals, stomach and right femur were observed compared to the NOVX and OVX groups (Table 2). The histopathological examination of liver, kidneys and lung of rats treated with different doses of *K. anthotheca* (125, 250 and 500 mg/kg BW) did not show differences compared to the NOVX or OVX groups (Fig. 9).

Table 2: Relative weights (mg/kg BW) of selected organs after 28 days of treatment with the aqueous extract of *Khaya anthotheca*.

Organs	SHAM	OVX	E2V	Diaz	Doses of KA (mg/kg BW)		
					125	250	500
Liver	31675 ±303	30571 ±410	31755 ±245	31342 ±295	32116 ±687	31811 ±515	30669 ±647
Kidneys	6405 ±144	5894 ±216	6083 ±130	5965 ± 078	6100 ±128	6020 ±117	6005 ±142
Lungs	6896 ±242	7182 ±474	7192 ± 390	6526 ±210	6945 ±228	6519 ±221	6939 ±249
Spleen	3273	3191	3182	3315	3451	3227	3327

	±318	±169	±139	± 054	± 053	± 057	± 067
Adrenals	379 ± 028	368 ± 025	381 ± 018	361 ± 024	352 ± 003	364 ± 024	361 ± 016
Stomach	11508 ±249	10736 ±237	11305 ±290	11559 ±364	10796 ±350	10512 ±102	11357 ±299
Right femur	2570 ± 053	2444 ± 040	2515 ± 029	2497 ± 030	2524 ± 021	2529 ± 052	2556 ±104

Data are expressed as mean ± SEM, n = 6 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. OVX; ααα $p < 0.001$ vs. NOVX (one-way ANOVA followed by Dunnett's test). # $p < 0.05$, ## $p < 0.01$ vs. NOVX (unpaired t-test).

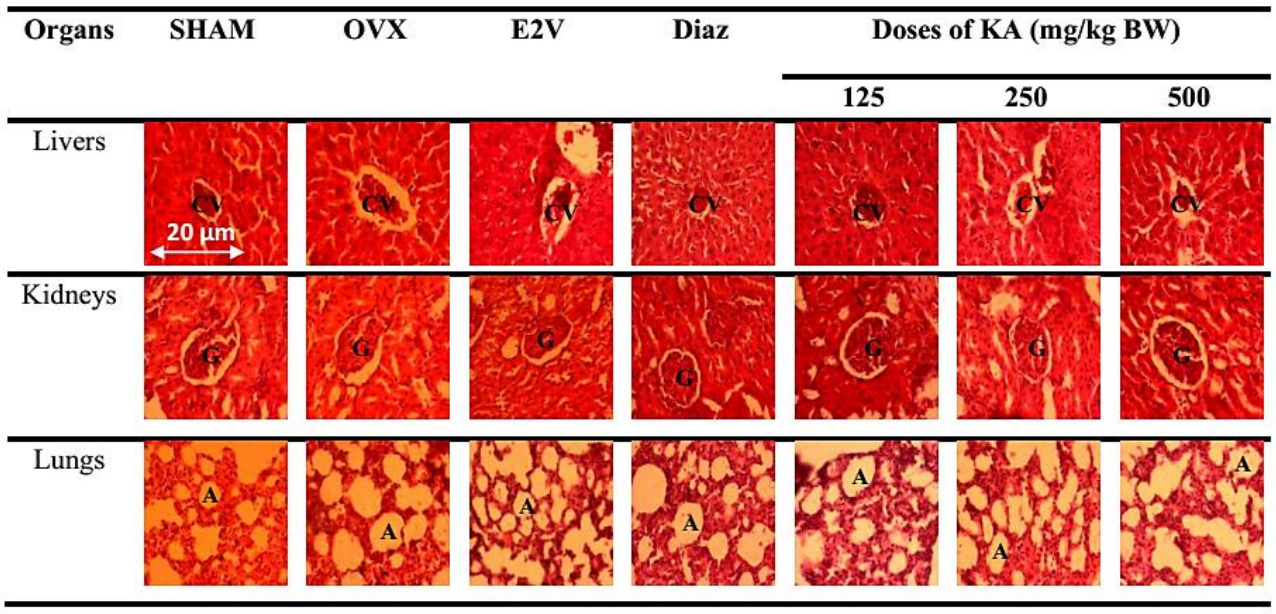


Figure 9: Effects of 28-days treatment with *Khaya anthothec a*extract on microstructure of selected toxicity organs (livers, kidneys and lungs) of ovariectomized Wistar rats.

Microphotographs (400 X). G: glomerular; CV: central vein; A: alveol.

Discussion

The elevated plus-maze (EPM) test was used to evaluate the anxiolytic effects of *K. anthotheca* extract on ovariectomized rats after 28 days of treatment. For the screening of anti-anxiety agents, the EPM is the most commonly test used to study anxiolytic response (Rodgers and Davlvi, 19971). In the literature, several authors showed that on the evaluation of the anxious parameters, an animal which explores the open arms is described as "not very anxious" and the animal who remains confined in the closed arms of the EPM is "anxious". In the same way, the increase of rearing and a decrease

of grooming and weight of fecal boli show a decrease of stress (Lister, 1999; Lund *et al.,* 2005; Oviedo *et al*., 2006). The results obtained in our investigation showed that the aqueous extract of *K. anthotheca* reversed the decrease of open arms exploratory of animal observe in ovariectomized animals. Compared to OVX group, the extract induced an increase of the percentage of entries into/time spent in open arms. It also induced a decrease of the percentage of entries into/time spent in closed arms. All these effects showed the anxiolytic effects of the extract in the EPM. Compare to OVX group, these anxiolytic effects of *K. anthotheca* extract are also materialize by a significant increase of rearing at the dose of 250 mg/kg BW and a significant decrease of grooming at all tested doses and weight of fecal boli at the dose of 500 mg/kg BW. The results obtained suggest that the extract could contain compounds responsible of the anxiolytic properties. Some author's show that compounds that are able to increase the open arm exploration in the EPM have anxiolytic activity (Walf andFrye, 2007; Adeyemi *et al*., 2010; Janaine *et al*., 2011; Ketcha *et al*., 2015). These anxiolytic properties could result from the action of these compounds on gamma amino-butyric acid (GABA) receptors complex (Xiu-Yan et *al*., 2007), like diazepam. Moreover, in ovariectomized rat, like 17β-estradiol, the steroids compounds present on this extract, can activate GABAA receptors on his steroids site fixation (Daendee *et al*., 2013) and the ER β that play a major role in the regulation of the anxiety in the brain (Lund *et al.,* 2005; Cheryl *et al*., 2010). It is well establishes in the literature that the anxious state appears by an increase in the body or rectal temperature (Marazziti *et al*., 1992). In our investigation, after 28 days of treatment, compare to OVX group, the *K. anthotheca* extract induce a decrease of the rectal temperature measure in rats. The extract at the dose of 500 mg/kg BW induced a significant decrease of this parameter. The literature reveled that estrogens play an important role in body temperature maintenance in women (Deecher and Dorries, 2007). The observed effects could justified the used of Khaya genus in the treatment of fever in African traditional medicine (Ojokuku *et al*., 2010) and these results suggest that *K. anthotheca* extracts could contain estrogenic compounds endowed with estrogenic properties.

At the end of 28-days treatment period, estrogen deficiency was accompanied with an atrophy of the uterine and vagina in OVX animals as expected. The treatment with *K. anthotheca* extract did not induce any significant modification in uterine but, regarding vaginal epithelial thickness, as well as E2V, the extract (at all tested doses) significantly increase the epithelial thickness. 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 (Njamen *et al*., 2013; Zingue *et al.,* 2013). This result suggests that *K. anthotheca* extracts could alleviate vaginal dryness experienced at menopause. The mammary gland, an estrogen target organ, was also studied. As well as E2V and compared to OVX group, the *Khaya anthotheca* extract at all tested doses induce an eosinophil secretion in lumen of alveoli. As showed by Zingue *et al*., 2013 and Njamen *et al*., 2013, estrogen-like substances reversed the blockage of eosinophil secretion in lumen of alveoli induced by ovariectomy. In humans, the gradual loss of estrogen contribute to weight gain, that has been reported in women during and after menopause (Tchernof *et al.*, 2000). Ovariectomy leads to a marked increase in body energy stores of the rat due to estrogen removal, which leads to increased energetic efficiency (Dagnault *et al*., 1996). Weight gain is the result of complex multifaceted processes, involving particularly the resistance to leptin, a decreased expression of adiponectin and excessive conversion of glucose into fat (Lima *et al*., 2005). In the present study, our results showed that after 28 days of treatment, ovariectomized animals' body weight significantively increased than that of the SHAM group. This increase in OVX animals' body weight is in accordance with the previous observations reported in the literature (Njamen *et al*., 2007 and Mvondo *et al*., 2011). It could be explained by deposits of fat subsequent to the alteration of the

energetic metabolism of lipids and increased abdominal adipose tissue caused by estrogen deficiency engendered by ovariectomy (Liu *et al*., 2009). As E2V, *Khaya anthotheca* extract at all tested doses inhibited weight increment induced by ovariectomy. This result suggests that this extract could contain compounds endowed with estrogenic properties and is in accordance with the observations of Naaz *et al.* (2002) who reported that estrogen reverses ovariectomy-induced body weight gain. The mechanism of action of estrogen and estrogenic substances in food intake is not yet well identified.

The results obtained on this work indicated that estrogen depletion induce anxiety. Several classes of compounds showed their anxiolytic property on the brain (Cheryl *et al*., 2010; Deepak *et al.*, 2013; Sarma *et al*., 2015). These anxiolytic properties of *K. anthotheca* extract could result from the action of some compounds funds in this plant like polyphenols (flavonoids). It is well known that flavonoids can activate $GABA_A$ receptors even in absence of GABA (Hanrahan *et al.,* 2011).

In the present study, to evaluate the safety of the extract, the acute toxicity and sub-acute toxicity profile of decoction were performed. In acute oral toxicity, a single oral administration of 5000 mg/kg BW of extract did neither induce mortality nor any toxicological symptoms in animals (body weight decrease, behavioral and respiratory patterns, somatomotor activities) and also after the 14-days post-treatment observation. Based on these results, *K. anthotheca* aqueous extract is classified in category 5 (OECD, 2001b) or unclassified (OECD, 2001b). Thus, the LD_{50} is estimated > 5000 mg/kg. In line with previous reports *K. anthotheca* extract would be considered as practically non-toxic extract (Ateba *et al.,* 2014; Awounfact *et al.,* 2016).

In sub-acute toxicological profile of the extract, the non variations in body weight after 28 days of treatment suggested that the administration of *K. anthotheca* did not alter the normal growth of animals. Relative weights of some organs is an indicator of toxic effects of drugs (Piao *et al.,* 2013). In this study, no significant variations in relative weights of liver, kidneys, lung, spleen, adrenals, stomach and right femur were observed compared to the NOVX or OVX groups. No changes in clinical signs induced by the treatment with the extract was observed. These observe effects could indicate a non-toxic effect of the *Khaya anthotheca* extract. The analysis of the microarchitecture of liver, kidney and lung confirm these observations.

CONCLUSION

This study was conducted to evaluate the anxiolytic, estrogenic and non-toxic effects of sub-acute treatment with decoction of *Khaya anthotheca* in ovariectomized Wistar rats. As results, 28-days of treatment with *K. anthotheca* extract exhibits anxiolytic properties in ovariectomized rats, induced a significant estrogen-like activity on some estrogen target organs and this extract show a broad margin of safety for a therapeutic use. The LD_{50} of the extract is estimated doses > 5000 mg/kg. These results suggest that *K. anthotheca* is endowed with anxiolytic and estrogenic effects, which justify its traditional use to manage central nervous system diseases.

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REFERENCE

[1] OO Adeyemi; AJ Akindele; OK Yemitan; FR Aigbe; FI Fagbo. *J. Ethnopharmacol.,* **2010**, 130, 191-195.

[2] E Amri; DP Kisangau. *J. Ethnobiol. Ethnomed.,* **2012**, 8, 1-9.

[3] SB Ateba; RV Simo; JC Mbanya; L Krenn; D Njamen. *Food Chem. Toxicol.*, **2014**, 65, 27-32.

[4] CF Awounfack; SB Ateba; S Zingue; MO Riepouo; D Njamen. *J. Ethnopharmacol.,* **2016**, 194, 169-178.

[5] M Casarrubea; MS Magnusson; V Roy; A Arabo; F Sorbera; A Santangelo; G Crescimanno. Temporal Pattenrns of Rodent Behavior in the Elevated Plus Maze Test. Eds. AJ Spink; F Grieco; OE Krips; LWS Loijens; LPJJ Noldus; PH Zimmerman, **2012**, p 350.

[6] SW Cheryl; AA Rebecca; AC Kathryn; J Yow-Jiun. *Inter. J. Women's Health,* **2010**, 2, 153-166.

[7] S Daendee; B Thongsong; S Kalandakanond-Thongsong. *Behav. Brain Res.*, **2013**, 246, 86-93.

[8] A Dagnault; Y Deshaies; D Richard. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.,* **1996**, 270, R199-R206.

[9] B David; J-L Wolfender; DA Dias. Phytochem. Rev., **2015**, 14, 299-315.

[10] DC Deecher; K Dorries. *Arch. Wom. Mental Health,* **2007**, 10, 247-257.

[11] PS Deepak; MW Kevin; P Peter. *Pharmacol., Rev.,* **2013**, 65, 1318-1350.

[12] JR Hanrahan; M Chebib; GA Johnston. *Br. J. Pharmacol.,* **2011**, 163, 234-245.

[13] MC Janaine; L Renata; G Lucas; CPM João; MWO Rúbia. *J. Ethnopharmacol.,* **2011**, 137, 1143-1148.

[14] WGJM Ketcha; S Djiogue; GF Zemo; NS Guemnag; D Njamen. *J. Ethnopharmacol.,* **2015**, 176, 494-498.

[15] WGJM Ketcha; GF Zemo; S Djiogue; CF Awounfack; D Njamen. *Int. J. Phytomed.,* **2017**, 9, 241-252.

[16] WP Lima; LC Carnevali; R Eder; LF Costa Rosa; EM Bacchi; MC Seelaender. *Clin. Nutr.,* **2005**, 24, 1019-1028.

[17] RL Lipnick; JA Cotruvo; RN Hill; RD Bruce; KA Stizel; AP Walker; I Chu; M Goddard; L Segal; JA Springer; RC Mayers. *Food Chem. Toxicol.,* **1995**, 33, 223–231.

[18] RG Lister. *Pharmacol. Ther.,* 1999, 46, 321-340.

[19] ZG Liu; R Zhang; C Li; X Ma; L Liu; JP Wang; QB Mei. *J. Ethnopharmacol,* **2009**, 123, 74-78.

[20] T Low Dog. *The Am. J. Med,* **2005**, 118, 98-108.

[21] TD Lund; T Rovis; WC Chung; RJ Handa. *Endocrinology,* **2005**, 146, 797-807.

[22] D Marazziti, A Di Muro; P Castrogiovanni. *Physiol. Behav.,* **1992**, 52, 393-395.

[23] MA Mvondo; D Njamen; S Tanee Fomum; J Wandji; V Günter. *Fitoterapia,* **2001**, 82, 939-949.

[24] A Naaz; M Zakroczymski; P Heine; J Taylor; P Saunders; D Lubahn; PS Cooke. *Horm. Metab. Res.,* **2002**, 34, 758-63.

[25] E Ngo Bum; S Soudi; ER Ayissi; C Dong; NH Lakoulo; F Maidawa; PFE Seke; LD Nanga; GS Taiwe; T Dimo; N Njifutie; A Rakotonirina; SV Rakotonirina; A Kamanyi. *Afr. J. Trad. Compl. Alt. Med.,* **2011**, 8, 130-139.

[26] D Njamen; S Djiogue; S Zingue; MA Mvondo; BN Nkeh-Chungag. **2013**. *J. Compl. Integr. Med.,* 10, 1-11.

[27] D Njamen; CB Magne Nde; ZT Fomum; JC Mbanya. *J. Compl. Integr. Med*., **2007**, 4, 1-6.

[28] FN Njayou; AM Amougou, TR Fouemene; MJ Njikam; R Sweha; B Bolling; EM José; FP Moundipa. *Cell Stress Chap*., **2015**, 20, 991-1000.

[29] OECD, Organization of Economic Cooperation and Development, 2001a. OECD Guidelines for testing of chemicals: Acute oral toxicity-Acute toxic class method. Test No. 423, adopted 22nd March 1996, and revised method adopted 17th December **2001**. OEDC, Paris.

[30] OECD, Organization of Economic Cooperation and Development, 2001b. Harmonised integrated classification system for human health and environmental hazards of chemical substances and mixtures. Chapter 2.1, adopted 14th August **2001**. OECD, Paris. (Chapter 2.1).

[31] SA Ojokuku; WO Okunowo; A Apena. *J. Med. Plants Res.,* **2010**, 4, 1126-1129.

[32] VM Oviedo; GG Milded; J Rincon; MF Guerrero. *Pharmacologyonline,* **2006**, 3, 342-347.

[33] A Petruczynik. *Centr. Eur. J. Chem.,* **2013**, 10, 802-835.

[34] Y Piao; Y Liu; X Xie. *J. Toxicol. Pathol.,* **2013**, 26, 29-34.

[35] J Pitkin. *Menopause Inter,* **2012**, 18, 20-27.

[36] R.J Rodgers; A Dalvi. *Neurosci. Biobehav. Rev.,* **1997**, 21, 801-810.

[37] P Sarma; M Borah; S Das. *Res. Pharm. Sci*., **2015**, 10, 206-213.

[38] V Scheid. *Compl. Ther. Med,* **2007**, 15, 54-68.

[39] A Tchernof; ET Poehlman; JP Despres. *Diabetes Metab.,* **2000**, 26, 12-20.

[40] N.J Toyang; M Nuwanyakpa; C Ndi; D Sali; WC Kinyuy. *Indig. Knowledge Develop. Monitor*. Regference PRELUDE: VT 08, **1995**.

[41] AA Walf; CA Frye. *Nat. Protoc.,* **2007**, 2, 322-328.

[42] WHO, World Health Organazation. Traditional medicine. Fact sheet 134. 2003-05. Archived from the original. **2008**, 07-28.

[43] C Wong; H Li K Cheng; F Chen. *Food. Chem.,* **2006**, 97, 705-711.

[44] W Xiu-Yan; Y Jing-Yu; W Jim-Hui; W Chun-Fu. *J. Ethnopharmacol.,* **2007**, 111, 613-318.

[45] GF Zemo; DL Amany; WGJM Ketcha; RF Oluwabusayo; S Djiogue; GT Taidinda; D Njamen;

O.J Olopade. *J. Ethnopharmacol.*. **2021**a, 275, 114099. Doi.org/10.1016/j.jep.2021.114099.

[46] GF Zemo; S Djiogue; EPF Seke; CA Pieme; AY Babiker; CF Awounfack; TRN Djikem; D Njamen. *J. Exp. Pathol*., **2021**b, 2, 174-181.

[47] S Zingue; D Njamen; J Tchoumtchoua; M Halabalaki; E Simpson; C Clyne; CB Magne Nde. *J. Pharmacol. Sci.,* **2013**, 123, 120-131.