Evaluation of Emmenagogue Activity of Aqueous Extract of Cajanus Cajan (Fabaceae) Leaves

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Abstract: The objective of this article is to evaluate emmenagogue activity aqueous extract of Cajanus cajan (fabaceae) leaves in rat. 100 g of leaves powder of Cajanus cajan were added 1 liter from distilled water and mixes it was carried to boiling then filtered. The filtrate was dried with the drying oven, the dried filtrate constitutes the aqueous extract (AECc). For the evaluation of emmenagogue activity, the rates in pseudogestation were used. These rates was obtained by coupling nulliparous and pubescent rates with vasectomized rats (rats made sterile by binding of vas deferens). AECc was managed with the amounts from 12.5 to 600 mg/kg of body weight, as well as the misoprostol with the amount of 2 mg/kg of body weight by oral way. The uterus and blood were taken for respectively the determination of the average weight and for the proportioning of progesterone. The results showed a variation of uterus weight as well as a variation of serum progesterone concentration at rate. AECc effect on the progesterone rate at the rate in pseudogestation gave a significant decrease (p<0.001) progesterone rate to amounts 100; 200; 400 and 600 mg/kg bw compared to control. As for amounts 12.5; 25; and 50 mg/kg bw, the aqueous extract decreased the progesterone rate in a nonsignificant way compared to the control. The aqueous extract of Cajanus cajan thus supports the menstruation with the amounts from 100 to 600 mg/kg bw and relieves of the painful rules to amounts 12.5 to 50 mg/kg bw.

Keywords: Cajanus cajan, Emmenagogue, Progesterone

1. Introduction

Several studies showed as increasing infertility in the world is caused as well at man as at woman by disturbers endocriniers, obesity, nicotinism, excessive alcohol consumption [1, 2] More specifically at woman, these causes would be of origin uterine, hormonal, tubo-peritoneale [3] Indeed, of the studies noted that in presence of one of these factors, there is a decline in the activity and the coordination of the axis hypothalamo-hypophysogonadic (HHG) responsible for regulation for reproductive system [2] These manifestations of the hormonal disturbances would result in menstrual disorders with knowing the amenorrheas (absence of menstruals, apart from pregnancy, at a woman able to procreate), dysmenorrheas (painful and repeated menses two or several times in month), the menorrhagia (prolonged and superabundant menstruals), metrorragies (uterine haemorrhage occurring apart from menstruals) [4].

Modern medicine would use non steroid anti-inflammatorys (pyclogenol, ibuprofene) and contraceptives oral (ulpristal, leuproreline, acetate of medroxyprogesterone) for treatment of menstrual disorders. Unfortunately, this treatment would present a certain number of disadvantages to
know of the gastro-intestinal disorders, a renal insufficiency, a hepatic toxicity, cardiovascular diseases, anemorheas, infertility [5, 6] However the emergency exit would be the alternative medicine by use of medicinal plants.

Thus, certain plants such as Combrerum racemosum Ficus umbellata Baphia nitida Desmodium adscendens are used like emmenagogues in alternative medicine [7] Indeed, emmenagogues plants are which contribute to the promotion and the regulation of the menstruation and thus treat many special disorders of the female system of reproduction. The emmenagogues can also be the anti-spasmodiques ones, relieving the uterine cramps and pains [8].

Cajanus cajan (fabaceae), a species of hardy perennial found in North and South-east of Côte d'ivoire [9]. The leaves of Cajanus cajan are used for food and traditional treatment several diseases in Africa, and more precisely in Côte d'ivoire against renal diseases, arterial hypertension, menstrual diabetes and disorders [10, 11] A phytochimic study of the sheets of Cajanus cajan revealed the presence of sterols and polyterpenes, polyphenols, flavonoides, tanins catechic, quinoid substances [12] conferred like emmenagogues in alternative medicine [7] Indeed, emmenagogues plants are which contribute to the promotion and the regulation of the menstruation and thus treat many special disorders of the female system of reproduction. The emmenagogues can also be the anti-spasmodiques ones, relieving the uterine cramps and pains [8].

2.2. Vegetable Material

The vegetable material used consisted of fresh leaves of Cajanus cajan These leaves were collected in the district of Azaguï located in the department of Agboville and the area of Agnebi-Tiassa. These leaves were identified and authenticated in National Center of Floristic (CNF) of the University Felix Houphouët Boigny (UFHB) of Abidjan (Côte d'ivoire). The number of herbarium allotted to this plant with the CNF is UCF10018.

2.3. Methods

2.3.1. Preparation of the Extract

The leaves of Cajanus cajan were dried out of the sun at room temperature for about two (2) weeks then crushed to obtain a powder of greenish color. For the preparation of the aqueous extract, 100 g of Cajanus cajan leaves powder were added to 1 liter of distilled water and the mixture was boiled for twenty minutes (20 min). The decoction was filtered twice on white cotton and once on filter paper Whatman N°3. The filtrate was oven dried at 40°C for two days [18].

2.3.2. Evaluation of the Emmenagogue Activity

Emmenagogue activity of aqueous extract of leaves of Cajanus cajan was carried out according to the method of [19] with some modifications. Nulliparous and pubescent rates were coupled with vasectomized male rats. Then with various stages of the experimentation, the uterus and blood were taken for respectively the determination of the average weight of the uterus and the proportioning of progesterone.

(i). Experimental protocol

Surgical operations dissection of the animals.

1) Realization of the vasectomy of the rats

Fifteen (15) adult of 202 ±4 g and male rats of 70 ± 3 days old were anesthesia with thiopental with the amount of 60 mg/kg bw by intraperitoneale injection. Their scrotums shaven then was disinfected with alcohol 90° and an opening of the scrotum or scrototomy was made to seek the vas deferens. This one being found, it was bound on both sides with wire with joining résorbable 2/0 then divided between the two bindings. Thereafter, the scrotum was sutured using the same wire and a disinfectant (betadine) was applied to this part. Lastly, the rats received 0.2 mL penicillin (antibiotic) by intramuscular injection. With their alarm clock, the rats received a analgesics amount (paracetamol 100 mg/kg bw). The bandage of the wound, on the level of the scrotum, was carried out each day with betadine and the rats received the same penicillin amount every two days until complete cure for one month.

2) Oil instillation at the rattes

To induce the decidualization, the old rates of 47 ± 3 days and weighing 170 ± 2 g, underwent sesame a right intra-uterine oil instillation. Those were put under general anesthesia with thiopental (60 mg/kg bw) and were placed in decubitus side. The right side of the rate was open in order to withdraw the uterus using a fine grip, and 300 oil µL of sesame were injected there. The uterus was then given inside and the peritoneum was sutured with wire resorbable. The skin was then sutured with wire not resorbable and the zone of operation asepticized with betadine.
A: Exposure of the vas deferens; B: Binding of the vas deferens; C Section of the vas deferens; D Cicatrization of the wound

Figure 1. Stages of realization of the vasectomy of the rat.

Figure 2. Sesame oil instillation in the uterine horn.

(ii). Vaginal smear technique

In order to determine the stage of the estral cycle, a vaginal smear was carried out on the nulliparous and pubescent weighing 120 ± 2 g and old rates of 40 ± 2 days.

1) Taking away of the vaginal cells

Cotton stem moistened of physiological liquid (NaCl 0.9%) was delicately introduced into the vagina of the rate without stressing it, then turned gently in the same direction until the appearance of a light resistance. Cotton was then withdrawn, then the taking away was spread out over a clean blade of microscope.

2) Colouring

Colouring was made with the methylene blue. A drop of methylene blue (2%) diluted to the 1/10ème was deposited on the blade carries object over which was spread out the vaginal taking away. Using a plate covers object, the preparation was covered, then the examination was made three minutes after the deposit of the drop so that the taking away is impregnated dye.

3) Blades examination

The examination of blades was made under optical microscope (G X 100) by counting a proportion of 200 cells from one end to another of blade. Thus the percentage of each cellular type made it possible to define various phases of oestrous cycle:

(a) the prooestrus (clean smear, 40 to 50% of eosinophilic cells and weak presence of leucocytes);
(b) the oestrus (clean smear, 60 to 90% of eosinophilic cells, not of leucocytes);
(c) dioestrus 3rd or metoestrus (dirty smear, 20 to 40% of eosinophilic cells, rather many leucocytes);
(d) dioestrus IΙ or anoestrus (very dirty smear, 10% of eosinophilic cells, very many leucocytes).

Protocol of test Effect of aqueous extract of Cajanus cajan on progesterone rate at the pseudo-gestante rate.

After realization of cervical smear, the rates presenting a stage oestrus were coupled with vasectomized males. Pseudo-pregnant females were identified by the presence of a vaginal stopper (day 1) for the continuation of the test. Vasectomized males were withdrawn at day 1.

The decidualization was induced by intra-uterine instillation of 300 µL of sesame oil to these pseudo-pregnant
females at day 4. Then at day 6; on the whole thirty-six (36) rates decidualized were selected and divided into nine groups of four to which it was managed by oral way, following substances:

1) Group I (Normal): rates decidualized having received water distilled under a volume of 10 mL/kg bw;
2) Group II (Positive): rates decidualized having received the misoprostol at the dose of 2 mg/kg bw;
3) Group III (AECc 12.5): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 12.5 mg/kg bw;
4) Group IV (AECc 25): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 25 mg/kg bw;
5) Group V (AECc 50): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 50 mg/kg bw;
6) Group VI (AECc 100): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 100 mg/kg bw;
7) Group VII (AECc 200): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 200 mg/kg bw;
8) Group VIII (AECc 400): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 400 mg/kg bw;
9) Group IX (AECc 600): rates decidualized having received aqueous extract of Cajanus cajan at the dose of 600 mg/kg bw;

(iii). Taking away

With various stages (8) of the experiment, for which the rates did not receive the aqueous extract; four (4) rates were sacrificed in a sequential way. Thus, the uteruses were taken on thirty-two (32) rates of which rates in proestrous (J0), at day 4 of pseudo-gestation (J4), at days 5; 6; 7; 8; 10 and 12 of the decidualisation (J5, J6, J7, J8, J10 and J12).

Therefore the uteruses were taken on the rates of groups I to IX at day 7. The wet weights of the uteruses were given.

(iv). Biochemical analyses

The rates were sacrificed by decapitation after anaesthesia to Thiopental (60 mg/kg bw). The samples of blood were taken in the dry tubes in order to proportion the biochemical parameters (hormonal). Blood was centrifuged with 3000 tr/min during 10 minutes and the serum was collected in a tube eppendorf then preserved at -20°C until the analysis of the hormonal parameters. The serum progesterone levels were given by using a test ELISA specific to the progesterone (progesterone rat/mousse ELISA IB79183).

2.4. Statistical Analyses

The data were analyzed by using an analysis of variance (ANOVA) followed by a test of Dunnett using the software Graph Pad PRISM 7.0. The level of significativity was given in comparison with the reference group and the group of reference. The statistical significativity was accepted for * P < 0.05; ** P < 0.01; *** P < 0.001 and # P < 0.05; ### P < 0.01; #### P < 0.001.

3. Results

3.1. Evolution of the Progesterone Concentration and the Weight of the Uterus During the Oestrous Cycle at the Rate

The curve of evolution of the progesterone concentration represented on figure 3 shows two (2) phases of which an ascending phase energy of day zero (J0) with the sixth (6è) day (J6) followed by a downward phase energy of the seventh (7è) day (J7) to the twelfth (12è) day (J12). Thus, compared to the average value of the progesterone concentration with J0 (90.71±4.36 ng/mL), the rate of the hormone knew a significant increase (p<0.01) with J4 (125.30±15.10 ng/mL) and very significant (p<0.001) with J5 (348.20±26.89 ng/mL), J6 (365.90±10.43 ng/mL) and J7 (255.00±10.80 ng/mL). However, from J8 with J12, the progesterone concentrations did not vary to a significant degree compared to J0 (Figure 3).

With the instar of the progesterone concentration, the average weight of the uterus of the rates knew a phase of increasing evolution of J0 with J5 followed by a decreasing phase of J6 in J12 (Figure 3). In addition, the average weight of the uterus remains very significant (p<0.001) in J5 (233.60±18.22 mg), J6 (229.70±14.26 mg) and J7 (185.20±7.40 mg) compared to the value of J0 (147.30±6.46 mg). In addition, the average values of the weight of the uterus of J8 with J12 are not significantly low compared to that of J0 (Figure 3).

3.2. Compared Effect of the Aqueous Extract of Cajanus cajan and Misoprostol on the Progesterone Rate at the Rate in Pseudogestation

The effect of the sheets of Cajanus cajan and misoprostol on the progesterone rate at the rate in pseudogestation is represented by Figure 4. This histogram shows that just like the misoprostol (2 mg/kg bw), the aqueous extract of Cajanus cajan to the amounts from 12.5 to 600 mg/kg bw induced a reduction in the progesterone rate compared to the witness (rates pseudogestantes having received distilled water). This reduction in the progesterone rate by the dose of AECc 100 mg/kg bw (173.20±5.26 ng/mL); 200 mg/kg bw (163.70±15.83 ng/mL); 400 mg/kg bw (157.20±10.27 ng/mL); 600 mg/kg bw (140.30±9.64 ng/mL) is very significant (p<0.001) following the example that induced by Misop (167.60±17.50 ng/mL) compared to the witness (255±10.67 ng/mL) (Figure 4).

The reduction in the progesterone rate induced by the doses of 400 and 600 mg/kg bw is more significant (p<0.05) that induced by the inhibiting substance of the action of progesterone of reference (Misop, 2 mg/kg bw) (Figure 4). The percentagles of reduction in progesterone compared to Misop for these doses (400 and 600 mg/kg bw) are respectively 6.20 and 16.28%.
**P < 0.01: significant difference compared to the control lot. ***P < 0.001: significant difference compared to the control lot.

Figure 3. Evolution of the progesterone concentration and the weight of the uterus during the oestrous cycle at the ratte.

Figure 4. Effect of the aqueous extract of Cajanus cajan and misoprostol on the concentration of progesterone at the 7th day of the pseudogestation at the ratte.

***P < 0.001: significant difference compared to the control lot (distilled water), ###p < 0.001: significant difference compared to the misoprostol, #p < 0.05 significant difference compared to the misoprostol, Control: lot treated with distilled water, AECc: aqueous extract of C. cajan, Misop: lot treated with misoprostol (2 mg/kg bw), AECc 12.5: lot treated with AECc (12.5 mg/kg bw), AECc 25: lot treated with AECc (25 mg/kg bw), AECc 50: lot treated with AECc (50 mg/kg bw), AECc 100: lot treated with AECc (100 mg/kg bw), AECc 200: lot treated with AECc (200 mg/kg bw), AECc 400: lot treated with AECc (400 mg/kg bw), AECc 600: lot treated with AECc (600 mg/kg bw).

4. Discussion

The study of emmenagogue activity was undertaken through the evaluation of the serum progesterone concentration which is a hormone of the regulation of the menstrual cycle. This hormone one of the biological markers is associated the fertility or female infertility related to the menstrual disorders. Rattes pseudogestantes were used in the study to exploit the intrinsic hormonal changes largely comparable with the human situation. In the present study, during the evolution of the weight of the uterus and concentration of progesterone during the oestrous cycle at the rate, the results showed a variation of the weight of the uterus as well as a variation of the progesterone concentration at the rate.

By taking of account the changes of the weight of the uterus and the variations of the rate of hormones (progesterone), we propose a correlation of the functional modifications of the menstrual cycle between the ratte and the woman. Thus the proestrus at the ratte could represent the proliferative phase at the woman. However, after the induction of the pseudogestation, the progesterone rates strongly increased, which indicates similarities with the phase of secretion (lutéale) at the woman. The fall of progesterone as from day 8 until day 12 could indicate the return to the proliferative phase at the woman. According to [19] in the mice reached of decidualized endometrium, the spontaneous fall of the endogenous progesterone levels is sufficient to induce processes similar to those of the human menstruations. Thus, the evaluation of the effect of the aqueous extract of the sheets of Cajanus cajan (AECc) on the progesterone rate at the ratte pseudogestante gave a significant fall (p<0.001) progesterone rate of the average doses to the high doses of 100; 200; 400 and 600 mg/kg bw compared to the witness. These results are similar to those of the antiprogestative molecule of reference used which is the misoprostol. As for doses 12.5; 25 and 50 mg/kg bw the aqueous extract decreased the progesterone rate in a no significant way compared to the witness. Indeed, with the strong amounts, Cajanus cajan would act as the misoprostol which is an analogue of the prostaglandin E1. The misopristol acts like natural prostaglandins [20]. That, the strong amounts of AECc, following the example misoprostol,
would interact with the receivers of prostaglandin of the uterus by blocking the receivers of progesterone causing a softening of the cervix and the contraction of the uterus, [21] what would cause the regression of the yellow bodies and the fall of the progesterone rate [22] This fall of progesterone supports the exfoliation of the layer functional calculus of endometor [23] Consequently, the effects observed in the present study suggest that the strong doses of AECc 100; 200; 400 and 600 mg/kg bw would degrade the functional layer of the endometor, contrary to low dose (12.5; 25 and 50 mg/kg bw) which would not have an effect on the aforementioned layer. This AECc fact, with high amount could be advised with the women who suffer from absence of menstruation (amenorrhea) and with weak amount with those which suffer from menstrual disorders such as the painful rules (dysmenorrhea) bus with low dose Cajanus cajan has a good analgesic activity [24] From its action of reduction in the circulating progesterone rate, Cajanus cajan would support the regression of the yellow body. This regression is at the base of the inhibition of the ovulation of the ovary systematic [25] causing elimination of the functional part of the endometor responsible for the menstruation.

A study undertaken by Zokore et al. made it possible to characterize in AECc the following chemical groups: sterols and polyterpenes, polyphenols, flavonoides, tanins, substances quinoid, saponosides. Some chemical compounds such as the quinoid substances, the tanins, the anthocyanes characterize in AECc the following chemical groups: sterols (amenorrhea) and with weak amount with those which suffer from the menstruation disorders such as the painful rules (dysmenorrhea) bus with low dose Cajanus cajan is a potential emmenagogue effect leaves of Cajanus cajan in order to isolate molecules with emmenagogue properties.

5. Conclusion

The study of emmenagogue activity of the aqueous extract of the leaves of Cajanus cajan showed that low dose of AECc lowers the progesterone rate slightly while the strong amounts caused a more marked fall of this hormone. The aqueous extract of Cajanus cajan thus supports the menstruation with doses 100 to 600 mg/kg bw and relieves of the painful rules to doses 12.5 to 50 mg/kg bw This investigation reveals a potential emmenagogue effect leaves of Cajanus cajan and supports its traditional therapeutic use in the treatment of the disorders of the menstruation. In a subsequent study, it would be wise to perform a fractionation of the aqueous extract of Cajanus cajan in order to isolate molecules with emmenagogue properties.

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References


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