Feeding castor beans to cyclic Nubian goats to induce luteolysis of the corpus luteum

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SUMMARY

In this study two experiments were designed to elucidate a probable luteolytic effect of castor beans fed to cyclic Nubian does. Experiment I: was designed to examine the effect of castor beans feeding on progesterone (P_4) profile during oestrous cycle in Nubian goats. Sixteen Nubian does were synchronized with two intramuscular injections of 125 µg $PGF_{2\alpha}$, 9 days apart; they were then grouped into two groups. Group I (n= 8) was fed 4 gm/animal castor beans on day 10 of the oestrous cycle. Group II (n= 8) was left to serve as control. Ten serum samples were collected from each doe at 48 hrs interval starting from the oestrus observed after synchronization, and assayed for P_4. The results of this experiment showed that castor bean feeding induced an abrupt change in P_4 profile 48 hrs after ingestion. A significantly ($P<0.001$) lower levels of P_4 were recorded on day 12 (0.27 ± 0.00 ng/ml) and day 14 (0.26 ± 0.00 ng/ml) of the oestrous cycle of castor bean fed does compared to those of the control group during the same days (5.6 ± 0.3 ng/ml and 5.5 ± 0.2 ng/ml) respectively. Experiment II was designed to compare the P_4 level during oestrous phase among does fed castor beans, does injected with $PGF_{2\alpha}$ and that of the control in experiment I. The 16 does in experiment I were give a rest for 2 months and they were observed for oestrous.

On day 9 of oestrous cycle they were intramuscularly injected with 125 µg/animal $PGF_{2\alpha}$ and grouped into two groups. Group I (n= 8) was fed the same amount of castor bean as in experiment I on day 10 of the oestrous cycle as in experiment I and group II (n= 8) was given a second dose of $PGF_{2\alpha}$. One serum sample was collected from each doe at time of oestrus and assayed for P_4 concentration. The results of this experiment showed that the serum P_4 concentration at oestrous phase in group I, group II and that of the control group in experiment I are similar ($P>0.05$). The values of P_4 levels recorded in group I, group II and the control of experiment I were 0.25 ± 0.01ng/ml; 0.39 ± 0.11 ng/ml and 0.19 ± 0.01 ng/ml, respectively.

It is concluded that castor bean is a strong luteolytic agent. Therefore castor beans must not be fed to pregnant animals whose pregnancy depends on corpus luteum.

INTRODUCTION

In the Sudan goats are known as selective grazers and browsers. However, selective grazing does not prevail in tropical pasture. During the periods of draught and pasture scarcity, which is not uncommon in the tropics, goats may be persuaded to consume what so ever stuffs in reach. The stuffs may be unpalatable, abortive toxic or even lethal to goats.

One of these stuffs is castor plant (*Ricinus Communis*). A flock of pregnant Nubian goats, which were grazed in pasture dominated by castor plant, ate castor seeds and aborted. This abortion was thought to be due luteolysis of corpus luteum, since a remarkable fall in the level of LH was reported in rabbits fed castor seeds (Abd Alla, 2000). Most of the information available about castor beans is about its toxic effect because it contains the toxic glycoprotein ricin. Scar information about the effects of castor beans on the reproductive system of ruminant is available (Elnaiem 2003).

In the light of the above incidence of abortion due to ingestion of castor bean, the objective of the current study was to document and elucidate probable luteolytic effects of castor beans fed to cyclic Nubian goats.

MATERIALS AND METHODS

Experimental animals:

Sixteen virgin Nubian does at the age of 1-1.5 year were used. Their average body weight was 24 kg. Two mature active bucks were kept leased with these does.

Animal Housing:

The animals were kept at El nisheishiba Experimental Farm at the University of Gizera Sudan (GUEF). The animals were kept in roomy pens made of local building materials. The leased bucks were kept outside the pens during night. During the day the leased bucks were allowed to join the flock in accordance with a preset program.

Hygiene measures:

Each doe was subcutaneously injected with 0.5 ivermectin (lvomec BD, Elnasar, Pharmaceutical Com. Egypt) to control internal parasites. The does were vaccinated against brucellosis. The vaccine was provided by the Animal Health office at the Gezira State, Animal Resources Head office at Meddani.

Herd running:

The flock was reared on the grass land of GUEF, and was managed by a trained stockman. The pasture of the grass land comprises of *Andropogona*, plots of forage sorghum, (*Sorghum vulgar* vr *Abu 70*. Browsing was provided by *Acacia nubica* and *Acacia proposes* shrubs. Every day the animals were taken to the pasture at 8:00 AM and flocked back at 5:00 PM. Drinking water was available from irrigation channels of the farm. The bucks were grazed with flock after been leased from front and hindquarters in a way providing easy movement for grazing and preventing mounting the does for breeding. This practice retained the social link between animals and provided effective teasing tool to detect oestrus. Each animal was provided with 250 gm of a supplementary ration composed of cotton seed cakes (33%), sorghum (*Sorghum vulgar* vr *fetarita*) grains (33%), wheat bran (33%) and Sodium chloride (1%).

Castor beans:

The castor beans (*Ricinns communis*) were conferred by the National Oil Processing Research Institute at University of Gezira as decorticated. The beans were then undecorticated and weighed. Two ounces of the undecorticated beans counted for 190 gm which

conferred a mean weight of approximately 400 mg/bean. The chemical composition was in accordance to that reported by Maiti *et al*. (1988). The fatty acids, ricinoleic acid (89.5%) linoleic acid (4.2%), oleic acid (3%), stearic acid (1%), palmatic acid (1%), dihydrostearic acid (0.7%), eicosanoic acid (0.3%) and lenoleic acid (0.3%) are among the chemical components of castor beans, in addition to the protein part of which the glycoprotein (ricin) is the important component.

Collection of Blood Samples:

Five millitres of blood were collected from the jugular vein of each doe by using disposable syringes. The samples were transferred to sterile test tubes and were allowed to clot. They were then centrifuged within 4 hours at a proximately 3000 r.p.m. for 10 minutes to separate the serum. The sera were transferred to Eppendorf micro test tubes and were kept frozen at -20ºC until assayed for progesterone (P_4).

Progesterone radioimmunoassay:

This was done at the Department of Radio isotopes of the Central Veterinary Research Laboratories, Soba. The P_4 in sera was assayed according to FAO/IAEA P_4 RIA protocol version 3.1 (1996). The detection limit (minimal detectable dose) of this assay is approximately 0.02 ng/ml.

Oestrous detection:

The leased bucks were allowed to join the flock during the day for the purpose of detecting does in heat. A trained personnel observed the oestrous signs three times a day for 30 minutes (7:00; 13:00; 18:00) the doe was considered in oestrus when it showed overt oetrous signs such as tail waggling, bleating, mounting others and/or accepted the attempt of the leased bucks to mount her or allowed other does to mount her (Mackenzie 1967).

Pretreatment to synchronize oestrous:

The oestrus was synchronized in all doe by prostaglandin F2 α ($PGF_{2\alpha}$). Each doe was intramuscularly injected with 125 µg of $PGF_{2\alpha}$ (Cloprestenol, Intervet Holland), 9 days later the does received a second dose of $PGF_{2\alpha}$ (Omar 2003). All the does expressed oestrous signs and were considered synchronized.

Experimental design:

Experiment I:-

This experiment was a one factorial design to test the effects of castor beans feeding to cyclic does on oestrous cycle and P_4 profile during oestrous cyclic. Eight synchronized does (Group 1) were fed 4 gm/animal castor beans on day 10 of the oestrous cycle (Elnaeim 2003). Ten serum samples were collected at an interval of 48 hours from each doe and were used for P_4 RIA as described above. Eight does (Group 2) were untreated to serve as control.

Experiment II:-

This experiment was a one factorial design to compare the P_4 level in serum at time of oestrus among does fed castor beans, does treated with $PGF_{2\alpha}$ and the control group in experiment I. The 16 does in experiment I were given a rest for 2 months and they were observed for oestrus. On day 9 of the oestrous cycle they were intramuscular injected with 125 μg of PGF2α and grouped into two groups and were observed for oestrus. On day 10 of the oestrous cycle group I (n= 8) was fed the same amount of castor beans as in experiment I. Group II (n=.8) was intramuscular injected with $PGF_{2\alpha}$. Blood samples were collected from each doe at time of oestrus (48 hr after treatment) and sera were separated and assayed for the P_4 level as described above.

Statistical analysis:

Data were subjected to one way ANOVA and probabilities at $p<0.05$ were considered significantly different. Data are presented as means ± SE. Two does in experiment II were excluded from the statistical analysis because they did not respond to $PGF_{2\alpha}$ treatment.

RESULTS

Experiment 1:-

As shown in fig.1, P_4 concentration from days 0 to day 10, which covers the metoestrous to midluteal phases, of the oestrous cycle of the castor bean fed does were comparable to those of the untreated control. No significant ($P>0.05$) differences were recorded in the P_4 level on the days of oestrous cycle in the treated and untreated control up to day 10 (the day of treatment). In both groups the P_4 concentration reached the beak on day 10. On day 10, P_4 concentrations of the castor bean fed does and the control were 6.8 ±

0.6 ng/ml and 6.5 ± 0.0 ng/ml, respectively. A sharp and abrupt fall in serum P_4 concentration of the castor bean-fed does was encountered on days 12 and 14 together with obvious oestrous signs. The P_4 concentrations on days 12 and 14 of the cycle of the castor bean-fed does were 0.27 ± 0.0 ng/ml and 0.26 ± 0.0 ng/ml, respectively. These values were significantly ($P<0.001$) lower than those of the control on the cross bonding days (5.6 ± .3 ng/ml and 5.5 ± 0.2 ng/ml, respectively). There after the serum P_4 concentrations of the treated does increased gradually to reach a peak value of 6.8 ± 0.2 ng/ml on day 20 of the cycle (10 days after castor bean feeding). The P_4 concentration of the control group remained high, up to day 16 of the cycle and sharp drops were recorded on day 18 and 20 (0.26 ± 0.00 ng/ml and 0.24 ± 0.00 ng/ml, respectively) together with obvious oestrous signs.

Experiment II:-

As shown in fig (2). The P_4 concentration at time of oestrous in castor bean-fed does and $PGF_{2\alpha}$ injected does were similar ($P>0.05$) to that of the control group in experiment 1 (0.25 ± 0.01 ng/ml; 0.39 ± 0.11 ng/ml and 0.19 ± 0.01 ng/ml, respectively). All the castor bean fed does expressed oestrous signs (n=8 does). While only 6 does, out of 8 does treated with $PGF_{2\alpha}$, showed oestrous signs.

DISCUSSION

In this study we reported for the first time that castor beans are luteolytic. This research provided and unambiguous evidence of a robust luteolytic action induced by castor bean feeding. This action was explained by exhibition of oestrous signs and the interruption of the P_4 profile during oestrous cycle of does fed the castor bean on day 10 of the cycle. The chemical composition of the castor beans is mainly fatty acids and the toxic glycoprotein ricin (Maiti et al. 1988). These fatty acids are ricinoleic, linoleic, oleic, stearic plasmatic, dihydroxy stearic, eicosanoic and linolenc acids. Most of these fatty acids are involved in the process of biosynthesis of prostaglandins (Goldyne 1988).

Accordingly, three possible pathways for the castor beans induced luteolysis were anticipated. These three possible pathways may act individually or jointly to induce the drastic luteolysis observed in this study. The first possible pathway is a cascade luteolysis induced by the lipid component of the castor beans. The vastly high content (80.9%) of the hyroxylated saturated fatty acid ricinoleic along with the relative abundant linoleic acid (4.2%) in addition to the considerable amounts of eicosanoic acid (0.3%) cooperate to make the core of the luteolytic complex. The abundant reserves of free linoleic acid in the castor beans will lead to biosynthesis of arachidonic acid and thus the formation of $PGF_{2\alpha}$ which leads to luteolysis of corpus luteum. However, the time that the biosynthesis of $PGF_{2\alpha}$ takes in goats is not known to us. The second pathway of luteolysis induced by the castor beans was through the action of ricin. The ricin may act in either of two ways: the first is by stress induction on the hypothalamic centers that regulate the GnRH and LH release. Acute stressors are known to interrupt the occurrence of normal GnRH-LH surges during follicular phase in ewes (Dobson et. Al. 2003). Also stressor reduced the response of pituitary gland to GnRH which was revealed by a reduction in LH level (Phagat et. Al. 1999). The castor bean feeding is known to reduce LH level in Rabbits (Abd Alla 2000). Accordingly we expected that the castor beans feeding induced stress and led to a reduction in LH level and luteolysis of the corpus luteum. The second pathway of action of ricin is directly on pituitary gland and corpus luteum. Ricin is known to suppress protein synthesis in the cell (Moldon and Stohs 1994). The biological damage to the luteal cells caused by ricin is expected to be through its direct action on luteal cells or its action on pituitary gland that impairs LH production. This is unlikely to occur because the dose used was far less than the lethal dose.

The third possible luteolytic pathway is that the castor beans contain other luteolytic agent (s), the possible candidate of which is $PGF_{2\alpha}$. This was thought because feeding the castor beans induced a rapid luteolysis. However, this assumption remains to be confirmed or rejected after a further analysis of the castor beans for $PGF_{2\alpha}$. Unfortunately this analysis can not be done in our laboratory.

In conclusion, the castor beans contain lutoelytic agent (s) which establish a short pacing and robust luteolytic process. Therefore, animals whose pregnancy depends on corpus luteum must not be fed castor beans or allowed to graze where castor plant prevails.

REFERENCES

Abd Alla, I. D. M. (2000). The effect of castor seeds on the fertility of female rabbits. *M. Sc. Thesis. Faculty of Medicine University of Geziera, Sudan.*

Dobson, H., Ghuman, S., Prabhakar, S. & Smith, R. (2003). A conceptual model of the influence of stress on female reproduction. *Reproduction* **125,** 151-163.

Elnaiem, W. M. (2003). The progesterone profile of the caprine oestrous cycle and luteolysis as induced by castor oil (Ricinus *communis*) seeds fed to cyclic goats (*Capra Hircus*). *PhD. Thesis. Faculty of Agricultural Sciences, University of Geziera, Sudan.*

FAO/IAEA. (1996). Progesterone radioimmunoassay protocol, version 3.1.

Goldyne, M. E. (1983). Prostaglandins and cyclic nucleotides. In: *Basic and Clinical Endocrinology.* Greenspan, F.S. and Forsham, P.H. (eds). Lange Medical Publication, Los Altos, California Ch.14. pp 368- 413.

Mackenzie, D. (1967). Goat Husbandry 2nd edition. Faber and Faber Ltd. 3. Queen Square London.

Maiti, S., Hedge, M. R.& Charropadhya, X. (1988). Hand book of Annual Oils Seed Crops. Oxford and IBH Publishing Co. Ltd.

Muldon, D. F. & Stohs, S. F., (1999). Modulation of ricin toxicity by biological active substances. Journal of. Applied Toxicology 14 (2), 81-86.

Phogat, P. B., Smith, R. E. & Dobson, H. (1999). Effect of adrenocorticotrophic hormone (ACT H^{1-24} on ovine pituitary gland responsiveness to exogenous pulsatile GnRH and Oestradiol and LH induced release in vivo. Animal Reproduction Science 55, 193-203.

Omer, N. N. (2003). Studies on the recrudescence of oestrous postpartum in Nubian goats. *M. Sc. Thesis. Faculty of Veterinary Medicine, University of Khartoum, Sudan.*

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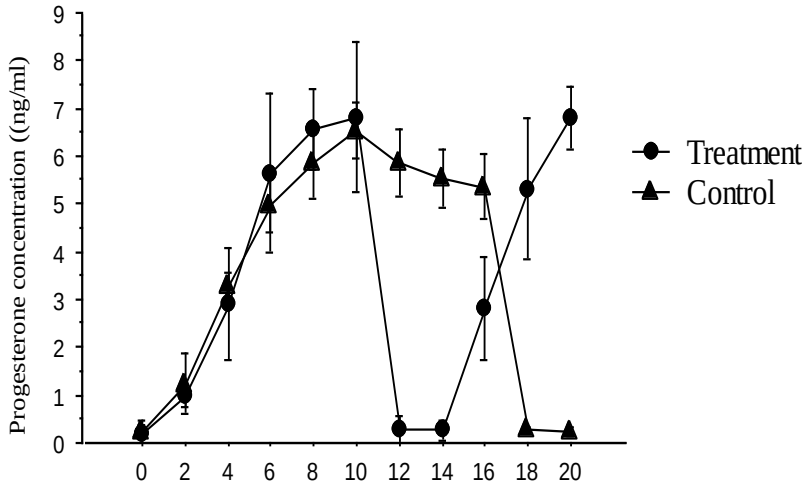

Fig. 1. Progesterone profile of Nubian does fed castor beans on day10 of the oestrous cycle
(● treatment) and untreated does (▲ control). Data were presented as means ± SE.
Each point represents a mean of 8 replicates. The vertical bars represent the SE.

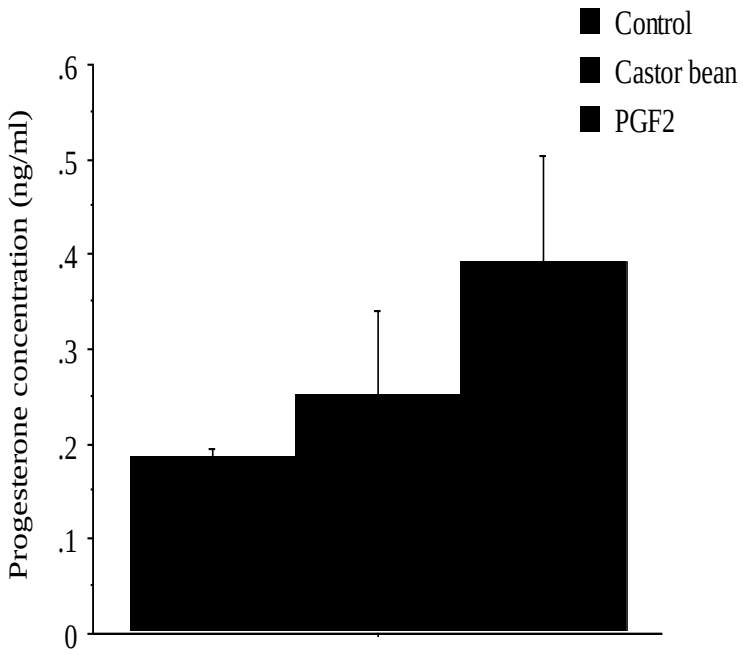

Fig.2. Mean progesterone concentration at oestrous phase in does treated with PGF2α
(PGF2), castor bean (castor) and control untreated does. Data were means ± SE.
Vertical bars represents SE

استخدام بذور نبات الخروع للماعز النوبي أثناء المرحلة اللوتينية من دورة الشبق يكسر الجسم الأصفر

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ملخص البحث:

أُجريت تجربتان في هذه الدراسة لمعرفة أثر تكسيري محتمل للجسم الأصفر عندما تعطى بذور الخروع للماعز النوبي أثناء المرحلة اللوتينية من دورة الشبق (Luteal phase).

التجربة الأولى أجريت لمعرفة أثر تغذية بذور الخروع للماعز النوبي أثناء المرحلة اللوتينية من دورة الشبق على مستوى البروجسترون (P_4 Profile). حقنت ست عشر معزة نوبية عضلياً بجرعتين (125 مغم × 2) من $PGF_{2\alpha}$ بينهما تسع أيام بغرض تزامن الشبق ثم وزعت على مجموعتين. المجموعة الأولى (عدد = 8 معزات) أعطيت بذور خروع بالفم (4 جرامات/حيوان) في اليوم العاشر لدورة الشبق. المجموعة الثانية (عدد = 8 معزات) لم تعطي بذور خروع واتخذت كشاهد. جمعت 10 عينات مصل دم من كل معزة كل 48 ساعة إبتداءً من ظهور الشبق بعد التزامن وتمت مقايسة هرمون البروجسترون إشاعياً في عينات المصل. لوحظ أن تغذية بذور الخروع تؤدي إلى تغير مفاجئ في P_4 Profile في الدم بعد 48 ساعة من تناوله. وقد انخفض مستوى البروجسترون معنوياً($p<0.001$) في الماعز المغذى المعالج . والتجربة الثانية أجريت لمقارنة تركيز الـ P_4 في مرحلة الشبق (Oestrous phase) بين الماعز المغذى ببذور الخروع وماعز محقون بـ $PGF_{2\alpha}$ مع تركيز P_4 الشاهد في التجربة الأولى. الـ 16 عشر معزة في التجربة الأولى أعطيت فترة راحة لمدة شهرين وتمت متابعة الشبق فيها. في اليوم التاسع لدورة الشبق حقنت عضلياً بـ $PGF_{2\alpha}$ (125 مغم/حيوان) وقسمت إلي مجموعتان. المجموعة الأولى (عدد = 8 معزات) أعطيت نفس كمية بذور الخروع كما في التجربة الأولى في اليوم العاشر من دورة الشبق التي تلت حقن $PGF_{2\alpha}$. المجموعة الثانية (عدد = 8 معزات) أعطيت جرعة أخرى من $PGF_{2\alpha}$ في اليوم العاشر من دورة الشبق التي تلت الجرعة الأولى. جمعت عينة واحدة من مصل الدم من كل معزة في اليوم الذي ظهر فيه الشبق وتمت مقايسة P_4 في هذه العينات. لوحظ عدم وجود اختلاف معنوي ($P>0.05$) في تركيز الـ P_4 عند مرحلة الشبق في المجموعة الأولى والثانية ومجموعة الشاهد في التجربة الأولى. وكانت متوسطات تركيز الـ P_4 في الدم في مرحلة الشبق في المجموعة الأولى والثانية والشاهد في التجربة الأولى هي 0.25 ± 0.01 نغم/مل ، 0.39 ± 0.11 نغم/مل و 0.19 ± 0.01 نغم/مل على التوالي. خلصت هذه الدراسة إلى أن بذور الخروع مكسر قوي للجسم الأصفر ، وعليه يجب عدم إعطائها للحيوانات الحوامل التي يعتمد الحمل فيها على الجسم الأصفر.