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Physiological Response Of Savanna Brown (SB) Does To Treatment With Mistletoe Extract (*Phragmanthera Nigritana*) And Clomid®.

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Abstract
Thirty six (36) nulliparous Savanna Brown does with mean body weight of 8.5 kg and aged 4-6 months were used to compare the physical and physiological changes that occur in SB does drenched with clomiphene citrate (Clomid®) and mistletoe (*Phragmanthera nigritana*) extract. The does were randomly assigned into three (3) treatment groups of twelve (12) does each. Each group was further subdivided into three (3) sub groups of split, full and double doses of extract. Those on the split dose of mistletoe extract were treated with 250 mg/kg twice in a day while those on full (500 mg/kg) and double dose (1000 mg/kg) were treated once a day. The second group were treated with Clomid®; split dose (0.35 mg/kg) twice in a day while those on full and double dose received 0.7 and 1.4 mg/kg administered once. The control groups were given distilled water. Normal routine management practices were employed and the does were managed intensively. Following treatment with the different doses of mistletoe, does exhibited reactions which vary from calmness, pasty ashy faeces, concentrated urine and reduction in weight which increased in the groups drenched with clomid®. Final weight showed significant difference between the clomid® and mistletoe treated group on split dose (8.40 ± 0.18 vs 10.83 ± 0.32) but both final weight and final pulse rate showed significant difference with does on clomid® and double doses (8.44± 0.29 vs 7.53 0.42 and 81.60 ± 0.55 vs 83.70 ±0.61) respectively, with better result in the group drenched with split doses (250 mg/kg) of Mistletoe extract. Split dose of 250 mg/kg administered twice in a day is recommended for use since the effects were not deleterious to the does.
Keywords: Physiological response, Savanna Brown does, mistletoe, clomid®, doses.

Introduction

Some of the means employed to enhance the productivity of livestock include improvement of livestock nutrition (Fasanya *et al.*,1992 a ,b) and the use of exogenous hormones like prostaglandin F_2 alpha (Alemede, 1995). However, the use of Clomiphene citrate (Clomid®) to improve productivity in animal is still very obscure. For thousand of years, knowledge of the herbs and wild plants that could increase productivity were the secret of the village wise women (Susan, 1999) but after the holocaust against the European wise women (the burning times) and the virtual extermination of native American medicine women, this knowledge virtually disappeared.

Many common plants can be used to influence reproductive and growth response of animals, these include red clover (*Trifolium pratense*), life root (S*enecio aureus*), wild carrot (*Daucus carota*) and wild yam (Dioscorea villosa). Others are mistletoe (*Phragmanthera nigritana*) and tropical nettle weed (*Fleurya aestuans*) (Akobundu and Agykwa, 1998; Alemede *et al*., 2014). Some of these grow in the wild while others are easy to cultivate but the issue of safe dosage levels of these plants and their role on the physiological response of animals have not been well documented.

Consequently, this study is designed to observe the various reactions shown by the savanna Brown does to different dosages of Clomid ® which is a standard exogenous hormones and mistletoe extract with a view of identifying a safe level of administration and creating a baseline data bank for future usage.

Materials and Method

Thirty-six (36) nulliparous Savana Brown does were used in this study to observe the physical and physiological reactions of does to Clomid ® and extracts of mistletoe obtained from lime tree. The does were aged 4 -6 months with initial body weight of between 6 -11kg. The animals were allowed a pre-treatment period of two weeks to enable them acclimatize during which they were checked to ensure that none was pregnant , labeled for identification and treated against ecto and endoparasites using 1 % ivomectin injection at recommended dose volume of 0.5 ml per 25 kg body weight (subcutaneously) and Albendazole bolus at a dose of 1 bolus per 50 kg

body weight (orally). Animals were also injected with long lasting broad spectrum antibiotics at a dosage level of 1ml /10 kg body weight. The does were randomly assigned into three groups of mistletoe, clomid® and the control and further subdivided into subgroups of split, full and double doses each with four animals per group as illustrated in the experimental layout (Table 1).

Mistletoe extract was prepared and the lethal dosage level was determined according to Organisation for Economic Cooperation and Development (OECD), guideline 423 (OECD, Guideline, 2000 a,b) Does in the mistletoe group were orally drenched with 250 mg/kg daily (split dose), 500 mg/kg daily (full dose) and 1000 mg/kg daily (double dose), the animals in the clomid® group received 0.35 mg/kg twice daily (split dose), 0.7 mg/kg daily (full dose) and 1.4 mg/kg daily (double dose) while those in the control group were given distilled water.
The animals were fed maize bran, groundnut hay, salt lick and concentrate *ad-libitum*. Drinking water was also supplied *ad-libitum*. Parameters measured were body weight, rectal temperature, pulse rate, physical appearance of animals, urine and faeces prior to and following administration.

Results and Discussion
Administration of 250 mg/kg (split dose) of mistletoe extract twice in a day (6.00 am and 6.00 pm), 500 mg/kg (normal dose) of mistletoe extract and 1000 mg/kg (double dose) in a single dose (6.00am) to Savanna Brown (SB) does, elicited some reactions among which were sudden calmness and voiding of concentrated urine within the first hour, pasty ashy faeces and reduced weight on day 1 and 2 respectively, followed by normal activities thereafter.
However, on administration of 0.35 mg/kg (split-dose) of Clomid ® twice in a day a (6.00 am and 6.00 pm) to the Savanna Brown (S.B) does, the animals urine turned amber colour within the first hour and reduction of weights was noticed by days 1 and 2. For the animals administered with full dose of 0.7 mg/kg Clomid ®, amber colour urine, weight loss at day 1, followed by normalized activities at week 2 were noticed. However, for animals drenched with 1.4 mg/kg Clomid ® (doubled doses), deep amber colour urine which cleared within 24 hours and a slight weight increase at day 3 were noticed. This result indicate a more debilitating reaction among the groups drenched with clomid®.
There was a significant change in the weight of the animal as indicated in table 2. The body weight was higher in animal dosed with mistletoe extract than those in control and those dosed with Clomid® this also agrees with Al-Amodi (2012) who reported that treating animals with doses of 50 and 100 mg/kg of mistletoe extract caused elevations in serum cholesterol and triglyceride levels and significant changes in the total protein levels thereby causing increase in body weight. Treatment with extract from *Viscum album* resulted in a reversal of high salt loading observed in high blood pressure but subsequently led to improved body weight of animals (Ofem *et al,* 2007). Mistletoe plant (V*iscum album*) has proved to have anti-lipidemic and antidiabetic potentials and could be useful in managing the complications arising from *Diabetes mellitus* such as hypercholesterolemia, hypertriglyceridemia, protein metabolism and anti oxidant status, 20 - 60 % *Viscum album* extract administered to the albino rats significantly ameliorate the altered protein contents of the diabetic animals ($P<0.05$) while 40 and 60 % concentration of the extract could bring the altered protein levels of the diabetic animals close to the control group (Onunogbo *et al.,* 2012).

The non significant ($P>0.05$) temperature changes observed in this study did not agree with Klopp *et al.* (1992) who reported that rectal temperature increased after each injection of mistletoe, this may be due to the oral route of administration used in this experiment, thus temperature and blood pressure regulation behave principally similarly to particular stages of mild fever and suggested a reaction of total organism to stimulus of the mistletoe extract injection. Meylan (2008) observed that the early morning rise in rectal temperature of 0.5 – 0.7 ^{0}C might be observed 24 – 48 hours after Clomid® administration and was due to central effect of progesterone secretion. Systolic blood pressure and heart rate were up regulated accordingly correlating to the basal body temperature of the test subjects (Klopp *et al,* 1992).

The final pulse rate of double dose was significantly higher in does under mistletoe than clomid® and control. However, Ofem *et al*. (2006) reported that, the crude mistletoe extract of *Viscum album* significantly lowered the blood pressure but had no effect on the heart rate in normotensive rats due to the vasopressive effect of mistletoe extract. Herb-mistletoe (2011) reported that most species of mistletoe extract administered to animals caused slowing as well as weakening of the heart beat, hypertension as well as narrowing the blood vessels in the skeletal muscles as well as the skin. This agrees with Eno *et al* (2004) who reported that basal heart rate levels in both normotensive and hypertensive animals were remarkably low, crude extract (5 -160 mg/kg) dose dependently reduced the rate of heart beat in normotensive rats while low doses (5 and 10 mg/kg) tested

produced about 3.23 % and 7.26 % depression of heart rate, respectively. Systolic blood pressure and heart rate were up regulated accordingly correlating to the basal body temperature of the test subjects (Klopp *et al*, 1992).

Conclusion

Results obtained from this study revealed that after treatment with the different doses of mistletoe, does exhibited reactions which vary from calmness, pasty ashy faeces, concentrated urine and reduction in weight which increased in the groups drenched with clomid®. Final weight showed significantly better result in the mistletoe treated group on split dose than those on split dose of clomid® (10.83 ± 0.32 vs 8.40 ± 0.18). Split dose of 250 mg/kg administered twice in a day is recommended for use since the effects were not deleterious to the does.

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Table 1: Experimental layout showing the total number of animals used and the groups.

Group	Treatment		
	Mistletoe	**Clomid ®**	**Control**
Split dose sub group.	4	4	4
Full dose sub group.	4	4	4
Double dose sub group.	4	4	4
Total	**12**	**12**	**12**

Table 2: Body weight, temperature and pulse rate of Savanna Brown does following the administration of different doses of mistletoe extract and Clomid ®

	Treatment			
	Mistletoe Mean±SEM	**Clomid ® Mean±SEM**	**Control Mean±SEM**	**LS**
Split dose				
Initial weight (kg)	9.50±0.87	9.50±0.87	10.00±0.00	NS
Final weight (kg)	10.83±0.32^{a}	8.40±0.18^{c}	9.33±0.14^{b}	*
Initial temperature(^{0}C)	38.93±0.15	38.85±0.29	39.15±0.35	NS
Final temperature(^{0}C)	38.33±0.17	38.33±0.16	38.04±0.19	NS
InitialPulse (beats/min)	82.00±2.00	75.50±2.63	84.00±4.00	NS
Final Pulse (beats/min)	81.88±0.41	81.85±0.67	81.33±0.72	NS
Normal Dose				
Initial weight (kg)	7.50±0.05	8.25±0.63	8.50±1.50	NS
Final weight (kg)	8.80±0.24	9.09±0.31	8.00±0.35	NS
Initial temperature (^{0}C)	38.65±0.38	38.53±0.26	38.85±0.15	NS
Final temperature (^{0}C)	38.41±0.12	38.18±0.12	37.83±0.21	NS
Initial Pulse (beats/min)	84.00±2.30	83.00±2.63	80.00±0.00	NS
Final Pulse (beats/min)	81.69±0.45	81.35±0.36	80.00±0.00	NS
Double Dose				
Initial weight (kg)	7.50±0.05	8.50±1.04	10.00±1.00	NS
Final weight (kg)	7.53±0.42^{c}	8.44±0.29^{b}	10.63±0.22^{a}	*
Initial temperature (^{0}C)	38.48±0.31	38.98±0.23	38.85±0.15	NS
Final temperature (^{0}C)	38.21±0.13	38.32±0.15	38.64±0.11	NS
Initial Pulse (beats/min)	83.00±1.91	79.50±2.63	76.00±4.00	NS
Final Pulse (beats/min)	83.70±0.61^{a}	81.60±0.55^{b}	80.90±0.33b	*

abc: Mean Values with different superscripts on the same row are significantly different (P<0.05)
LS: Levels of significance
*: Significant different (P<0.05)
SEM: Standard Error of Mean
NS: Not significant (P<0.05)

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