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Abortifacient activity of *Sorghum bicolor* sheath extract and its effects on selected reproductive hormones in female Wistar rats

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ABSTRACT: About 90% of women have been reported to become anaemic in pregnancy in sub Saharan Africa and India. *Sorghum bicolor* sheath (SBS) has been scientifically proven to be effective in anaemia treatment, but the safety of its consumption during pregnancy is unknown. Thirty-nine (39) female rats of weight 160 ± 20 g were used for this study. The test group was daily administered 800 mg/kg body weight aqueous extract of SBS. The animals were sacrificed in batches on days 7, 14 and 19. Selected hormonal assay and haematology were carried out, the uteri of the pregnant rats were observed for changes and the organ-body weight ratio calculated. The results showed a significant reduction ($p < 0.05$) in the weight, progesterone, oestrogen and number of implantations in the test group by day 14 of the experiment. A significant increase ($p < 0.05$) was observed in the haematological parameters of the test group. There was also a 50% resorption noticed in the pregnant rats administered the extract compared with the control rats, with no significant difference ($p > 0.05$) in the organ-body weight ratio of the liver. It was concluded from this study that *Sorghum bicolor* sheath extract possess abortifacient activity.

Keywords: *Sorghum bicolor* sheath, Abortifacient, Pregnancy, Hormones, Haematology, Steroids

Introduction

The use of herbal medicine has been on the increase in many developing countries [1]. These herbal remedies are used due to their cost effectiveness and ease of access [2]. The percentage of women depending on herbal medicine for their healthcare needs in developing countries is about 80% [3]; therefore restricting its use by pregnant women has been a herculean task.

Due to the percentage of women in developing countries dependent on herbal products for management of pregnancy associated symptoms such as anaemia, headache, nausea etc [4, 5,6], its use has become inevitable for some women. It has been reported that about 10% of birth defects are attributed to agents such as the environment, drugs, nutritional or biologic factors exposed to during pregnancy [7].

The belief that herbal remedies are safer for use in pregnancy than orthodox medicine has been discovered to be inaccurate in some cases [3]. The unregulated use of plants by pregnant women as mineral and vitamin supplements, which are required for foetal growth, have in time past posed potential hazard to the foetus [8]. It has also been reported that about 70% of birth defects with unknown aetiology might be attributable to some of these plant materials due to their chemical components [3]. More so, some of these herbs have saponin, polyphenols and other antinutrients that may prevent absorption of micronutrients [9, 10].

Sorghum bicolor is a cane like grass, with a height of up to 6 meters and large branched clusters of grains [11]. It is a grain mainly grown across Africa, America, and Asia especially dry and hot areas [12]. It is called 'Jowar' in India, 'Bachanta' in Ethiopia, "Oka pupa" in the Southern region of Nigeria, 'Karan dafi' in the Northern part of Nigeria [13-15].

The sheath of *Sorghum bicolor* possess anti-anaemic [16-20], anti-inflammatory [21-23] and antioxidant activities [24, 25], high mineral, vitamin B_{12} contents [11], as well as high ratio of omega-6 to omega-3 fatty acids [26, 27]. *Sorghum bicolor* sheath plays beneficial role in treatment of neuropsychiatric symptoms associated with depression, memory deteriorations and psychotic manifestations [28-31].

Despite the use of *Sorghum bicolor* sheath as a proven anti-anaemic agent, its safety in pregnancy is yet to be ascertained hence the reason for this study. This study therefore determined the effect of *Sorghum bicolor* sheath extract on selected female hormones, organs and implantations in pregnant rats.

Materials and Methods

Plant Material and Authentication

Sorghum bicolor sheath was obtained from a farm in Ilora, Oyo State, authenticated at the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria and the voucher number UILH/001/1043 was issued.

Laboratory Animals

Thirty nine (39) Female rats weighing 140-180 g were obtained from the Animal House, Central Research Laboratories, University of Ilorin, Kwara State. This study was approved by the Ethical Committee of the University of Ilorin with approval no: UERC/ASN/2015/215.

Enzyme Assay Kits

Progesterone, Oestrogen, Luteinizing hormone and Follicle Stimulating hormone assay kits were products of Calbiotech Inc., California, USA.

Other Reagents

Analytically graded reagents were used and prepared with distilled water.

Preparation of Plant Extract

The sheath was dry cleaned with a cloth, air dried and pulverized. The pulverised sample was then infused in water at 100^0C for 10 minutes. Buchner funnel was used in filtering the extract, and the filtrates concentrated at 40^oC using a SHA-C1water bath [32].

Secondary Metabolites Screening

The secondary metabolites present in the Sheath were quantitatively determined using the methods of [33-37].

Animal Grouping and Experimental Design

Thirty nine (39) Female albino rats were housed in cages during the period of the experiment. They were fed on rat chow all through the period and allowed free access to water. Male rats were put in cages of the female rats in the ratio 1:2 for 3 days and the females were observed for vaginal plugs. After confirmation of pregnancy, they were administered 0.5ml of 800 mg/kg bodyweight of the extract daily throughout the period of pregnancy. Thirteen (13) rats were sacrificed in batches on days 7, 14 and 19 which represented the three trimesters of pregnancy in rats (Figure 1). The following hormonal analysis were carried out on the serum of the rats: Progesterone, Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Oestrogen and also observed were; number of live dams, number of implantation sites and number of resorption sites [38].

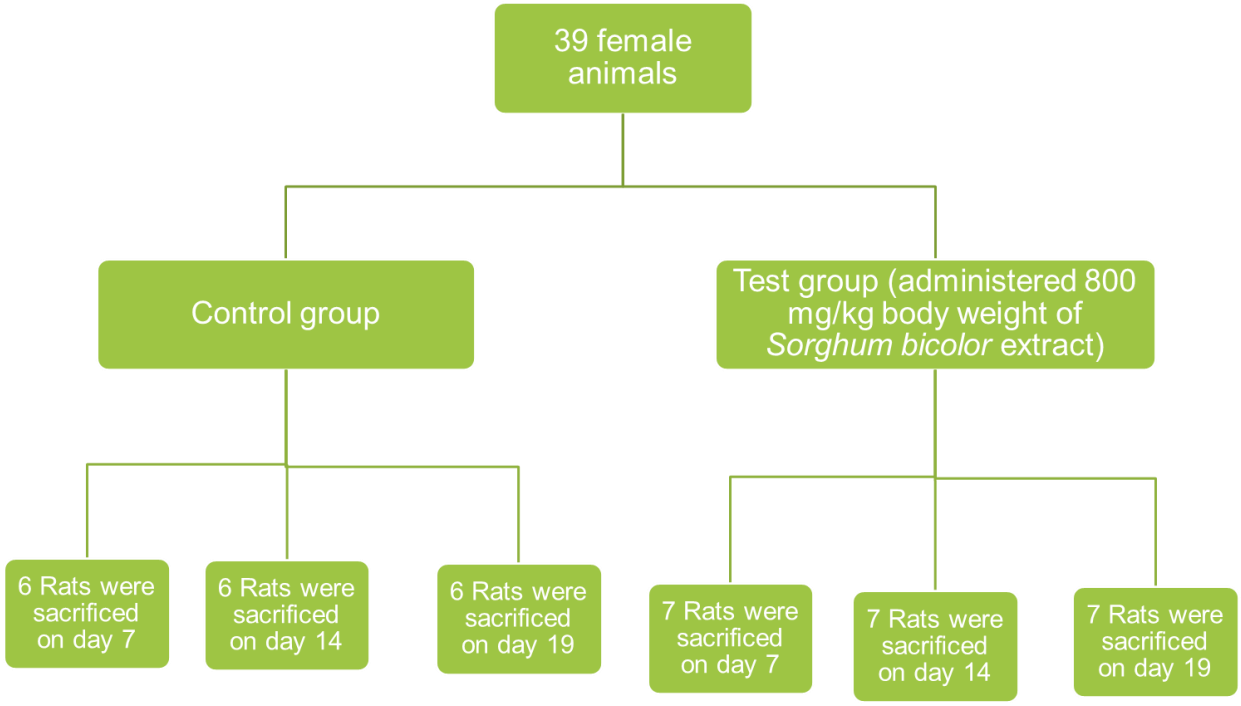


Figure 1: Animal grouping of pregnant female rats administered 800 mg/kg body weight of *Sorghum bicolor* extract

Biochemical assay

Serum was obtained from the blood by centrifuging for 15 minutes using a refrigerated centrifuge (Eppendorf centrifuge, AG, model: 5804R) at 3000 rpm. Progesterone, oestrogen, luteinizing and follicle stimulating hormonal assays were carried out on the serum based on Enzyme linked immunosorbent assay (ELISA) principle, a microplate reader was used to read the absorbance at 450 nm wavelength. The Sysmex KX 21N haematology analyzer was used for the haematological indices of the pregnant rats.

Organ-body Weight Ratio

The liver and kidney of individual rats were removed and weighed using a weighing balance.

The organ-body weight ratio was expressed as a percentage and calculated using the formula:

$$\%\text{Organ-body weight} = \frac{\text{weight of the organ}}{\text{Weight of the whole animal}} \times 100$$

Statistical Analysis

SPSS package version 21 and GraphPad prism 6 were used. Values obtained were expressed as mean ± standard error of mean (SEM). The values were subjected to Analysis of Variance (ANOVA), as well as Duncan's Multiple Range Test, to determine statistical significance. Differences were considered significant at $p < 0.05$.

Results and Discussion

Aqueous extract of *Sorghum bicolor* possess steroids, flavonoids, alkaloids, saponins and terpenoids (Table 1) which have been reported to be responsible for the abortifacient activity observed in rats administered aqueous extract of *Alchornea cordifolia* roots [39]. Flavonoids have been reported to possess antifertility activity [40, 41], alkaloid like constituent extracted from aqueous *Graptophyllum pictum* were reported to be responsible for the suppressant effect on uterine contraction and high anti-implantation activity observed [42]. Steroids from *Indigofera trifoliata* leaves were suspected to be responsible for the antifertility effects observed [43]. These metabolites were also present in *Sorghum bicolor* sheath and might be responsible for its abortifacient activity.

Table 1: Secondary metabolites of *Sorghum bicolor* sheath

Constituents	Concentration (mg/l)
Phenols	2.19 ± 0.01
Saponins	2.95 ± 0.01
Flavonoids	2.21 ± 0.01
Cardiac glycosides	2.88 ± 0.02
Terpenoids	0.65 ± 0.03
Steroids	0.40 ± 0.02
Tannins	0.91 ± 0.07
Alkaloids	2.85 ± 0.01
Phlobatannins	0.26 ± 0.02

The extract of *Sorghum bicolor* sheath had higher saponin, cardiac glycoside, alkaloid, flavonoid and phenolic contents, the metabolite with the highest concentration was saponin while phlobatannins had the lowest concentration.

The significant decrease in weight of the rats administered *Sorghum bicolor* sheath (as shown in Figure 2) could be due to the foetal loss and resorption observed in this study. This is in tandem with the report of Oguejiofor *et al*. [44] who treated rats with aglepristone and reported a similar pattern of weight loss. This is especially true because there was no significant difference in the percentage liver body weight ratio of the test and control groups and this is suggesting that the *Sorghum bicolor* extract had no toxic or deleterious effect on the liver of pregnant animals (Table 2). Although there was a significant increase in the kidney of the test group, this might be due to an increase in the secretory function of the kidney thereby leading to hypertrophy as reported by Awotunde *et al.* [45].

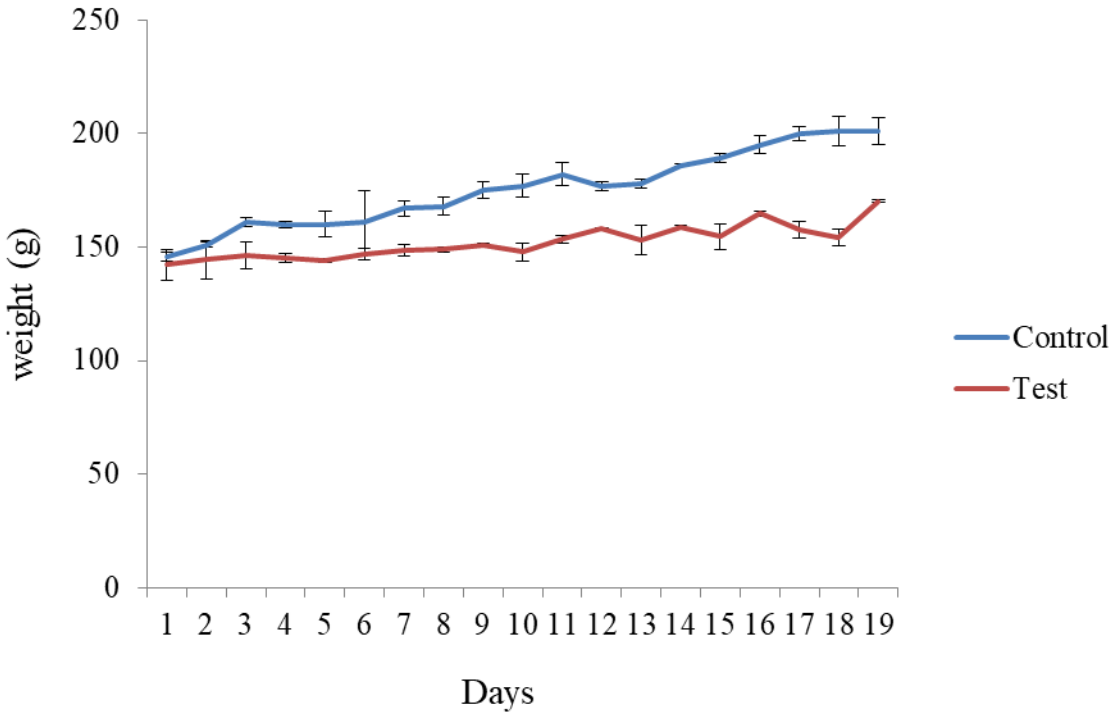


Figure 2: Growth pattern of animals during the period of pregnancy
(Test group was administered 800 mg/kg body weight of *Sorghum bicolor* aqueous extract)
The growth pattern of the animals during the period of pregnancy is presented in Figure 2. There was a significant increase ($p < 0.05$) in the weight of the animals in the control group all through the period of the experiment when compared to the test group. There was no observed significant difference ($p > 0.05$) in the weight of the control and test group as at day 6 (first trimester). Between days 8 to 13 (second trimester), there was no observable significant increase ($p > 0.05$) in the weight of the test group when compared to the control group. A major fluctuation in weight was observed in the test group starting from day 14 up till day 19 (third trimester) when compared to the control group.

Table 2: Effect of Sorghum bicolor extract administered at 800 mg/kg body weight on pregnant rats

Groups	**Liver**	**Kidney**
Control	4.06 ± 0.35^{a}	0.47 ± 0.02^{a}
Test (administered 800 mg/kg bwt)	3.80 ± 0.02^{a}	0.70 ± 0.04^{b}

Haematological indices of pregnant rats administered 800 mg/kg bodyweight of aqueous extract of *Sorghum bicolor*

The haematological indices of pregnant rats upon administration of 800 mg/kg body weight of aqueous extract of *Sorghum bicolor* is shown in Table 3. The significant increase in the haematological parameters of the treated group when compared to the control group might be adduced to the haematinic effect that has been reported for *Sorghum bicolor* (Table 3) and also that the extract does not have any toxic effect on the red blood cells [19, 46]. This haematinic effect was also reported for some already

established anti-anaemic plants such as *Magnifera indica, Theobroma cacao, Alchornea laxiflora* with the effect attributed to the bioactive compounds present in the plants [47, 48, 49].

Table 3: Haematological indices of pregnant rats administered 800 mg/kg bodyweight of aqueous extract of *Sorghum bicolor*

Haematological Indices	**Control (no treatment)**	**Test group (administered 800mg/kgbwt of extract)**
WBC x10^3/µL	6.70 ± 0.20^a	18.58 ± 3.16^b
RBC x10^6/µL	6.01 ± 0.01^a	7.00 ± 0.21^b
HGB (g/dL)	9.20 ± 0.10^a	10.80 ± 0.63^a
HCT (%)	35.40 ± 1.40^a	40.35 ± 1.58^a
MCV (fL)	63.05 ± 1.95^b	57.60 ± 0.75^a
MCH (pg)	15.20 ± 0.20^a	15.40 ± 0.42^a
MCHC (g/dL)	25.15 ± 0.15^a	26.75 ± 0.64^a
PLATELET x10^3/µL	864.00 ± 4.00^a	876.00 ± 79.08^a

Values are means of 5 determinations ± SEM. Values with different superscripts across the row are significantly ($P < 0.05$) different. WBC: white blood cell; RBC: Red blood cell; HGB: Haemoglobin; HCT: Haematocrit; MCV: Mean corpuscular volume; MCH: Mean cell haemoglobin; MCHC: Mean cell haemoglobin cell.

There was a significant increase ($p < 0.05$) in the white blood cell and red blood cell count of test group when compared with the control group (table 3), and a significant decrease ($p < 0.05$) in the mean cell volume of the test group when compared with the control. There was no significant difference ($p > 0.05$) in the haemoglobin, haematocrit, mean cell haemoglobin, mean cell haemoglobin cell and platelet of both control and test groups.

For oestrogen, there was no significant difference ($p > 0.05$) in the concentration of the rats in the control and test groups as at day 7 and day 14 post implantation. At day 19 post implantation, there was a significant decrease ($p < 0.05$) in the concentration of the test group when compared to the control group. For progesterone, there was no significant difference ($p > 0.05$) in the concentration of the test and control groups as at day 7, by day 14 post implantation, there was a significant reduction ($p < 0.05$) in the test group compared to the control group while there was no significant difference at day 19 post implantation for both groups.

For luteinizing hormone**,** there was no significant difference ($p > 0.05$) in the concentration in the rats at days 7, 14 and 19 post implantation in both test and control groups.

For follicle stimulating hormone, there was a significant decrease ($p < 0.05$) in the concentration in the test group at day 7 when compared to the control group, however at days 14 and 19 post implantation, there was no significant difference ($p > 0.05$) in both groups as shown in Figure 3

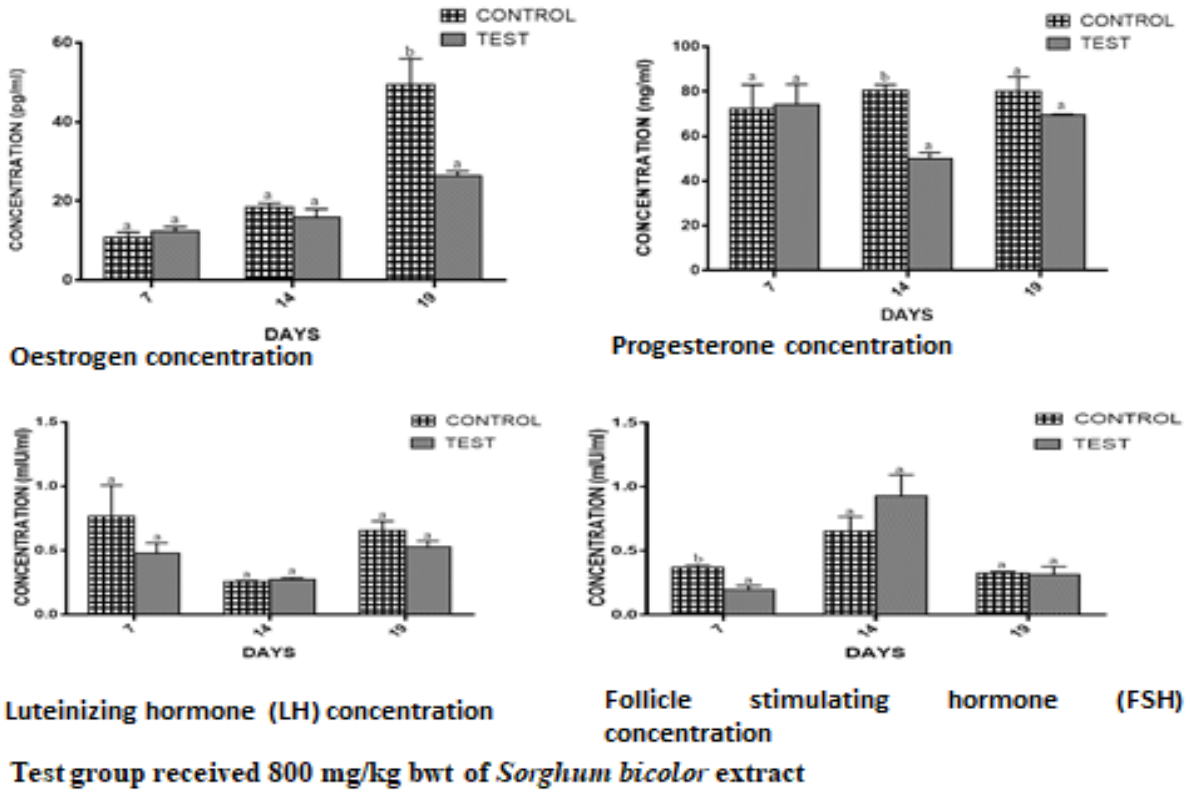


Figure 3: Effect of aqueous extract of *Sorghum bicolor* sheath on selected hormonal concentration of pregnant rats

Table 4: Percentage organ to body weight ratio of pregnant rats following oral administration of aqueous extract of *Sorghum bicolor* for 19 days

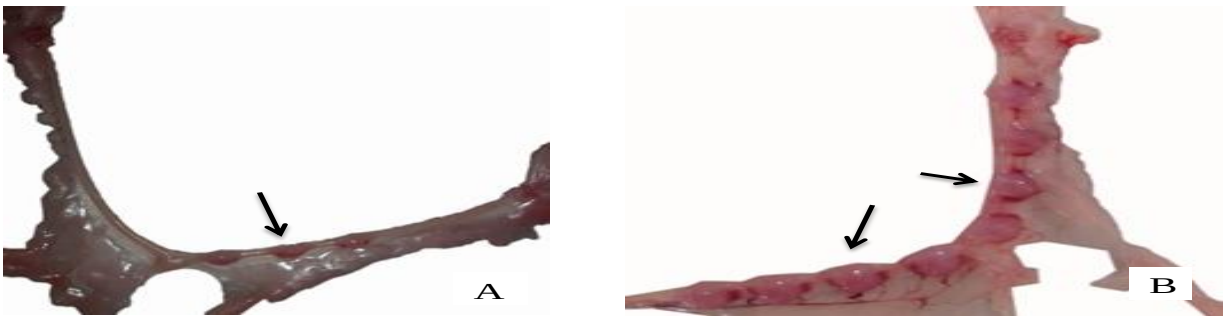
Days post implantation	Pregnancy Parameters	Control Groups	Test group
0	Body weight (g)	145 -180	145 – 180
7	No of implantation	13	20
	% pregnant	80	42.86
	% resorption	0	14.3
14	No of live foetus	28	9
	% pregnant	60	16.7
	% resorption	0	50
19	No of live foetus	24	8
	% pregnant	70	20
	% resorption	0	60
	% abortifacient	Nil	55.6

In the 7 days post implantation, there were more implantations in rats pregnant in the test group compared to the control group while more rats had implantations in the control group (80%), there was no resorption site noticed in the control group while the test group had 14.3% resorption. By the 14th day post

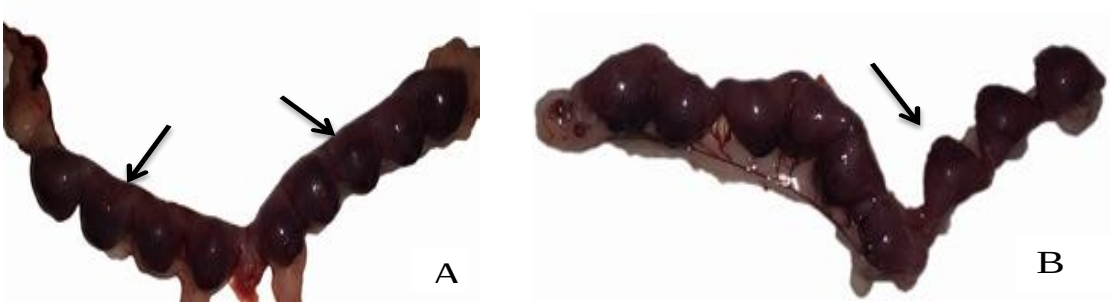
implantation, there was a drastic decline in the number of live foetus and a 50% resorption in the test group while the control group had 60% pregnancy. In the 19th day post implantation, there was 60% resorption in the test group and an overall 55.6% abortion rate in the test group while none was observed in the control group as shown in Table 4.

Photograph of Rat uterus following oral administration of 800 mg/kg bodyweight aqueous extract of *Sorghum bicolor* for 7, 14 and 19 days

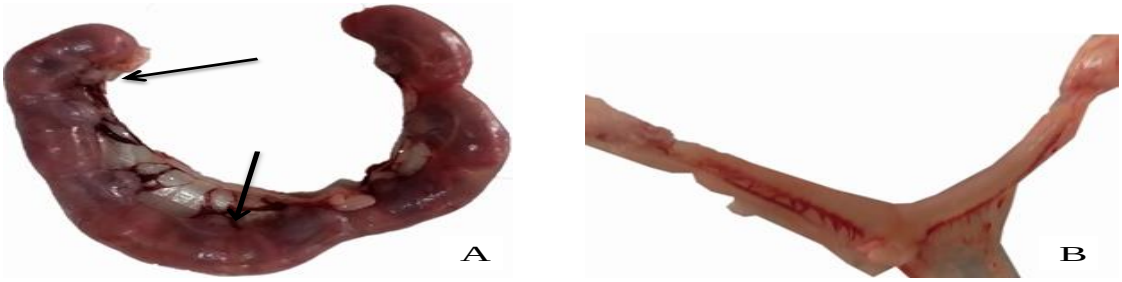
Plate 1 shows images of rat uteri and foetuses upon administration of 800 mg/kg body weight of *Sorghum bicolor* extract.



7 days



14 days



19 days

Plate 1: Photograph of rat uterus following oral administration of 800 mg/kg body weight aqueous extract of *Sorghum bicolor*

A: control group; B: group administered *Sorghum bicolor* extract (Black arrow is showing implantation in uterus of animals)

At day 7, there were more implantations (black arrow) observed in the test group compared to the control group (Plate 1).

By day 14 of pregnancy, there were malformations noticed in the uterus of the test group (B) when compared to the control group (A). The foetuses (black arrow) in group B were not having same formation as that of the group (A).
At day 19 of pregnancy, the rats in the control group (A) were found to be pregnant with no sign of resorption or abortion while the rats in the test group (B) had swollen uterus filled with uterine fluid as shown in Plate 1.

Oestrogen stimulates the growth of the uterine lining causing it to thicken and also stimulates granulose cell proliferation during follicular development. The significant decrease observed in the test group when compared to the control group on day 19 (Figure 3) can be adduced to a distortion of the uterine lining caused by *Sorghum bicolor*. This result agrees with the abortifacient effect observed in the pregnant rats and supports that reported by Oguejiofor *et al*. [44] in which a reduction in serum oestrogen levels were observed post-implantation upon treatment of rats with aglepristone.

Effect of aqueous extract of *Sorghum bicolor* sheath on selected hormonal concentration of pregnant rats

Figure 3 shows the effect of *Sorghum bicolor* sheath extract on hormonal concentration of pregnant rats. Progesterone has been reported to prepare the body for conception and pregnancy and also helps in pregnancy maintenance [44, 50, 51]. Decrease in the concentration of progesterone is a suggestion of impaired endometrial function, disrupting normal secretion of protein required to nourish implanted fertilized egg and prenatal development [51, 52]. There was a reduction in the progesterone level on the 14^{th} day of pregnancy and this suggests impairment in endometrial function leading to loss of implantation. This corroborates the observation in previously reported results. The abortion started as from the 14^{th} day hence the observed reduction in hormonal level. Decline in progesterone level has also been reported to possibly be due to toxic effects of plants directly on the corpus luteum, leading to spontaneous abortion [53]. There was no significant difference in the concentration of both groups at day 19^{th}, probably due to the steroids present in the plant thereby acting as a progesterone antagonist on the uterus. This plant has been found to possess steroids and the report is in agreement with that of Oguejiofor *et al*. [44] but not in agreement with that of Sharaibi and Afolayan [51] who reported that steroids were responsible for the amelioration of the damage caused to endometrium function thus promoting reproduction. There was no significant difference in the concentration of luteinizing hormone (LH) in both control and test rats. LH stimulates secretion of sex steroids from gonads and also ovulation of matured follicles [54]. The extract of *Sorghum bicolor* had no effect on the luteinizing hormone concentration suggesting that it did not affect ovulation [55]. This might also be responsible for the absence of significant difference observed in the oestrogen and progesterone levels. Follicle stimulating hormone (FSH) is essential for gonadal development and maturation at puberty, gamete production and also stimulates growth and maturation of ovarian follicles [56]. The significant increase in the control group at day 7 might be that the extract reduced the stimulating effect on the ovarian follicles [51, 52]. This might have contributed to the effects observed in the steroidal hormones.

There was an increase in the value of percentage resorption over continuous use of the plant, at 7 days it was 14.3% which rose to 60% at the 19^{th} day (table 4). This could be adduced to an imbalance in oestrogen-progesterone concentration, as any disturbance in the levels can cause abortion, resorption or lack of implantation [57]. This is suggesting that a prolonged use of *Sorghum bicolor* extract has abortifacient activity, as a 55.6% abortifacient activity was reported in this study which was found to be a little lower than that reported for *Amaranthus viridis* (57.14%) [58] and a bit higher than that reported for the aqueous extract of *Rumex studelli* (53%) [58].

As at day 7 of pregnancy, the implantation sites were visible and more than those observed in the control suggesting that the plant helped in reducing pre-implantation losses. However, by day 14 of pregnancy, there were malformations observed in the uterus of the test group compared to the control group and these malformations were also noticed in the weight of the rats in the test group which started

to fluctuate (plate 1). By the 19th day of pregnancy, the uterus of the pregnant rats in the test group had no foetus, it was filled with uterine fluid suggesting resorption of the foetuses and hence abortion. Studies carried out on *Rumex studelli* roots [59] also revealed post implantation losses. This was adduced to the presence of alkaloids, steroids, flavonoids and phenolics which were also present in *Sorghum bicolor* sheath and also corroborated by previous researchers [60, 61]. *Sorghum bicolor* has been reported to be used in addition to slice root of *Baphia nitida*, soaked with potash as an abortifacient in Lagos State [62]. The result obtained from this study therefore lends credence to its use as an abortifacient.

Conclusion

The present study lends credence to the anti-anaemic properties of *Sorghum bicolor* sheath extract. However, *Sorghum bicolor* sheath caused post implantation losses in pregnant animals, an observation attributable largely to the steroidal content of the extract. There was no sign of toxicity on the blood, liver and kidneys of the pregnant rats. Although anaemia is often associated with pregnancy, aqueous extract of *Sorghum bicolor* sheath is not recommended for use during pregnancy.

Conflict of Interest

The authors have declared no conflict of interest.

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