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Original Article

Phytochemical screening and anti-implantation activity of *Asparagus africanus* root extract in female Sprague–Dawley rats

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ABSTRACT

Asparagus africanus Lam., Asparagaceae, is used traditionally as medicinal plant for treatment of various gastrointestinal disorders and for birth related applications. This study aimed to evaluate anti-implantation potential, screening for bioactive phytochemicals and to determine its toxicity. Thirty healthy rats were distributed into five groups (n = 6). Pregnant rats were orally administered vehicle and aqueous extract *A. africanus* at three different doses thrice daily for seven days. Misoprostol 300 μg/kg bw was used as positive control. All rats were laparotomized 24 h after the last dose and number of live fetuses, implantations and resorption sites were enumerated, and ovaries were harvested for histopathology. The phytochemical analysis was carried out using LC/MS. Acute toxicity was investigated, the animals were randomly grouped into five groups (n = 3); control, four different doses of aqueous extract *A. africanus* at a single dose treatment and rats were observed for 14 days. Prenatal study demonstrated that 300 mg/kg bw of extract and misoprostol were significantly increased the percentage of anti-implantation as compared to untreated rats. Histopathology of ovaries showed a dose dependent toxicity. LC/MS revealed the presence of steroidal saponins; asparasaponin II, sarsasapogenin, spirostans, and stigmasterol. The mean weight gain of rats treated with 2000 mg/kg bw of aqueous extract was significantly reduced ($p = 0.032$) compared to control group. In conclusion, the aqueous extract *A. africanus* has anti-implantation effect in female rats and is safe up to 2000 mg/kg bw. In addition, it contains some potential steroidal saponins, which could be used to explain its anti-implantation activity, however this finding needs further pharmacological studies to confirm the antifertility activities.

Introduction

It is extremely obvious that plants play cogent role in the well-being of humans and animals. Hundreds, if not thousands of plant materials have been reported to possess medicinal properties owing to their phytochemical constituents. In addition, many plants have been traditionally explored as remedy for various pathologic conditions such as: burning sensation, fever, nephropathy, hepatopathy, bladder irritation, throat infections, tuberculosis, cough, bronchitis, leucorrhoea, hemorrhoids, hypertension, abortion, and cardiac problem (Warrier et al., 1993). One of such medicinal plants is *Asparagus africanus* Lam., Asparagaceae, a climbing shrub, which has been used traditionally to treat diverse ailments such as: peptic ulcer, diarrhea (Hutchinson et al., 1963), headache, backache, stomach pain and for easing childbirth (Maroyi, 2011). *A. africanus* has also been used for other non-therapeutic usage such as food and ornamental purposes (Ribeiro et al., 2010). Despite all these aforementioned uses of this plant, there are still insufficient data regarding its abortifacient and toxicological effects.

Geremew et al. (2006) showed that the root of *A. africanus* possess hormonal properties which can modulate the reproductive function of the experimental rats and suggested that the contraceptive activity of *A. africanus* needs further exploration to elucidate the bioactive constituents. In line with this, a previous study has been reported two compounds from the roots of *A. africanus* namely, spirostanosides and furostanol glycoside using combining MS with two-dimensional NMR technique (Asfaw et al., 1999). Furthermore, the structure of isolated compound, sapogenin, from *A.*

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africanus has been elucidated by combining X-ray crystallography with MS and 2D NMR (Oketch-Rabah et al., 1997).

Antifertility of *A. africanus* extracts has been studied in rats and a single dose of 300 mg/kg bw of leaves and roots extracts showed the possible effect of the plant in hormonal signaling pathway which may be responsible for certain reproductive function (Geremew et al., 2006). *Asparagus africanus* has been reported to ease childbirth and expulsion of placenta after birth (Hamill et al., 2003), whereas its aqueous extract had inhibited conception in a dose-dependent manner in Sprague–Dawley (SD) rats (Okidi et al., 2019).

Owing to the fact that chromatography couple to mass spectrometry method is a direct and fast analytical approach for identification of phytocomponents (Goyal, 2014), this study aimed to screen and identify the major phytochemicals in the aqueous extract of *A. africanus* root using LC/MS. Subsequently, to determine the *in vivo* anti-implantation activity and acute toxicity of the aqueous extract of *A. africanus* in SD rats.

Materials and methods

Chemicals and reagents

Misoprostol was purchased from Cayman (Michigan, USA). Ethanol and methanol were of HPLC grade and obtained from Fisher Scientific (Illkirch, France). Haematoxylin, bluing reagent and eosin were obtained from Leica Microsystems (Milan, Italy). All other chemicals were of analytical grade and obtained mainly from Merck and Friedemann Schmidt, Germany.

Plant material

Asparagus africanus Lam., Asparagaceae, was collected between September 2015 and January 2016 from Sindiri village in Damaturu; local government area of Yobe State, Nigeria. The location is having the bearing of long 11^0, 44″, 52 s, N. latitude 11^0, 57″, 35 s, E. The various plant parts were obtained from a healthy plant. The plant was authenticated by taxonomist at Institute of Biological Sciences, Faculty of Science, University of Malaya with voucher specimen No. KLU:48696. The voucher specimen was deposited in Rimba Ilmu Herbarium. The root parts were washed with distilled water and air dried at room temperature. The dried roots were ground with an electric blender into a fine powder to pass through a 0.2 mm sieve (BS 410 Endecott's Ltd., London).

Plant extraction

Approximately 50 g of the fine powder of the roots were extracted using 500 ml of ultra-pure water for 24 h in temperature control wrist shakers at 20 °C (Dunn et al., 2004). The extract was filtered using Whatman filter paper no. 4. The extract was kept in a freezer at −20 °C for a day before being evaporated for 48 h in a vacuum using a freeze dryer (Eyela FDU-1200, Japan). The obtained yield extract was kept in a fridge at 4 °C until used for further analysis.

Anti-implantation experiment

The study was conducted using a healthy female Sprague–Dawley rats weighing 160–200 g obtained from Animal Experimental Unit, Faculty of Medicine, University of Malaya. All experimental procedures were carried out according to the approval of the ethics committee for animal experimentation, Faculty of Medicine, University of Malaya (Ethics Reference no.: 2015-180505/PHAR/AEI).

The anti-implantation activity of aqueous extract of *A. africanus* was evaluated according to the method described by Salhab et al. (1999), with slight modification. Briefly, female rats were paired overnight with the male that has passed sexual behavioural testing (ratio 1:1) in plastic cage that provide free access to food and water. Pregnancy was confirmed by the presence of vaginal plug and spermatozoa in the vaginal smear that can be observed under the light microscope (Marcondes et al., 2002). The first day of pregnancy was considered as day 0 of gestation (G0). The pregnant rats were randomized into five groups, consisting of six animals each (n = 6). On G1 to G7, pregnant rats were orally administered vehicle, 300 μg/kg bw misoprostol as positive control and *A. africanus* extracts at three different doses (50, 150, and 300 mg/kg bw) thrice daily. The rats were weighed weekly, and all rats were laparotomized 24 h after the last dose (day 8), under anesthesia (50 mg/kg ketamine/5 mg/kg xylazine), and the following parameters were recorded: number of implantations and number of resorption sites (Salhab et al., 1999). Gravid ovaries from the females exposed to the different treatments were harvested and proceeded for histopathological examination (Guillaumon and Couto, 2013). Anti-implantation activity was expressed as percentage using the following formulae according to Geremew et al. (2006):

$$\textit{Anti-implantation activity} = \frac{\text{No. of implant in control} - \text{No. of implant in test}}{\text{No of implant in control}} \times 100$$

Histopathology of ovaries

At the end of the study, the ovaries were examined for gross pathological changes and the ovary tissues were collected and fixed in 10% formalin for 12 h. The samples were harvested by cutting 4–5 μm thickness and stained with haematoxylin and eosin stain (Kadagi et al., 2014). Changes in follicles and corpora lutea organization and luminal epithelial cell height were investigated and compared with the control untreated rats (Kadagi et al., 2014; Shaikh et al., 2015).

Liquid Chromatography/Mass Spectrometry (LC/MS Q-TOF)

Agilent technologies with LC/MS:6550 model iFunnel LC/MS-Q-TOF; po.05.01(B5125.3), and MS range (*m/z*): 100–1700 were used for this analysis. Aqueous extract (5 mg) from the root was redissolved in 5 ml of HPLC grade methanol and filtered using polytetrafluoroethylene (PTFE) syringe filter with 0.2 μm pore size. A column zorbax eclipse plus C18 from Agilent technologies with a dimension of (4.6 m × 100 mm, 3.5 μm) was used for the peaks separation, the initial temperature of the column oven was 32.8 °C and it was ramped up to final temperature of 34.4 °C. The MS and MS/MS scan rate were both at 1 spectra/sec with initial pressure of 115.1 bar to final pressure of 114.2 bar at the end of the run.

The instrument was operating at a flow rate of 0.5 ml/min with injection volume of 10.0 μl, and needle wash time of 3 s. The LC grade mobile phase comprises of two solvents: A (0.1% Formic acid in Milli-Q water) and B (100% methanol HPLC grade), with graded elution system. The total run time was 20 min, with a post run time of 10 min (Alali et al., 2008; Agilent, 2015; Han et al., 2015).

Identification of phytoconstituents

Chromatograms and spectrums obtained were identified using database of National Institute of Standards and Technology Library (NIST). Mass Spectrum Search Interpreter version 11 database was used for possible chemical identification by comparing the spectrum, and *m/z* of unknown compounds with the range of known

phytoconstituents stored in the NIST library, also by molecular feature algorithm in the software package to elucidate the compounds. The probable names of compounds were identified.

Acute toxicity study

Healthy female Sprague-Dawley rats were randomly distributed into five groups (n = 3) and fed with a standard rat pellet and water *ad libitum*. The animals were housed in plastic cages at 25 ± 2 °C temperature and 50 ± 10% humidity, in a standard light/dark cycle (12 h light/12 h dark cycle) and left for one week to acclimatize to these conditions. Furthermore, administration of extract, blood and organs sampling as well as all surgical procedures were conducted with humane care according to good practice guide to the administration of substances and removal of blood, including the routes and volumes (Diehl et al., 2001).

The rats were fasted for 3–4 h before oral administration of aqueous extract of *A. africanus* to the groups, but water was allowed *ad libitum*. Group I served as normal control, gavaged with distilled water; groups II, III, IV, and V were orally administered single dose of crude extract dissolved in distilled water at 5, 50, 300 and 2000 mg/kg bw, respectively (Kadagi et al., 2014). Wellness parameters of animals were observed continuously during the first half hour after dosing and periodically for the next 24 h and then daily for 14 days. All observations were systematically recorded.

At day 15, the animals were fasted overnight prior to being sacrificed under ketamine (150 mg/kg) and xylazine (15 mg/kg) anesthesia. Blood from each animal were collected for biochemical analyses including, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALT), blood urea nitrogen (BUN), uric acid (UA), creatinine (Cr) and total protein (TP). The remaining whole blood was collected in EDTA tubes for haematological parameters (Halpern et al., 2015). Liver and kidney were harvested from all rats and examined for gross pathological changes and representative samples were collected in freshly prepared 10% buffered formalin for histopathology examination. Sections of 4–5 μm thickness from liver and kidney were stained with haematoxylin and eosin (H&E) and examined under the microscope (Kadagi et al., 2014).

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for data with normal distribution (body weight, ALP, ALT, AST, TP, Cr, urea, uric acid, HGB, RBC, MCH, RDW, WBC and platelets), while Kruskal–Wallis was used for data with non-normal distribution (HCT, MCHC and MCV). IBM SPSS software version 22 (Chicago, USA) was used to analyze all data. Statistical significant was considered at ($p < 0.05$).

Results

Evaluation of anti-implantation activity of Asparagus africanus *aqueous extract (AEAA) in female SD rats*

The results obtained are summarized in Figs. 1–3. The average weight gain for normal control group was lower than treated groups. Significant increase in weight gain was observed in rats treated with 300 μg/kg bw misoprostol ($p < 0.009$), and 150 mg/kg bw *A. africanus* ($p < 0.025$). For 50 mg/kg bw *A. africanus* and 300 mg/kg bw *A. africanus*, the difference was not significant when compared with normal control. 150 mg/kg bw *A. africanus* may be equivalent to effective dose that favour the growth requirement of the animals. Overall the highest mean weight gain throughout the study period was observed in misoprostol treated group (Fig. 1).

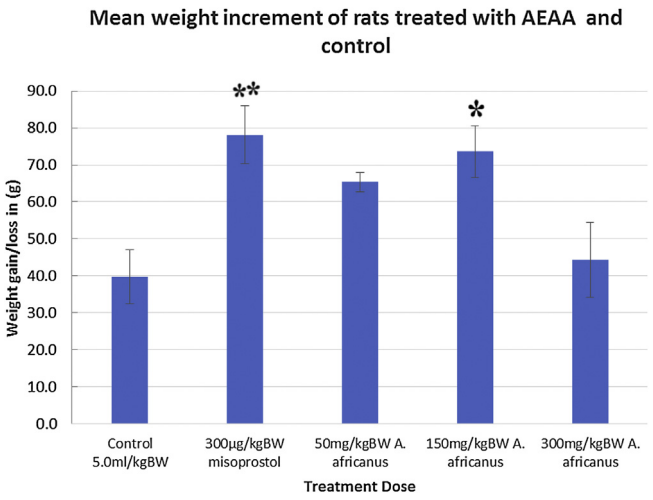

Fig. 1. Effect of *Asparagus africanus* on weight gain for pregnant rats. Values are expressed as mean ± SEM, (n = 6), $p^*< 0.05$, $^{**}p < 0.005$ vs. control. kgbw, kg per body weight; DW, distilled water.

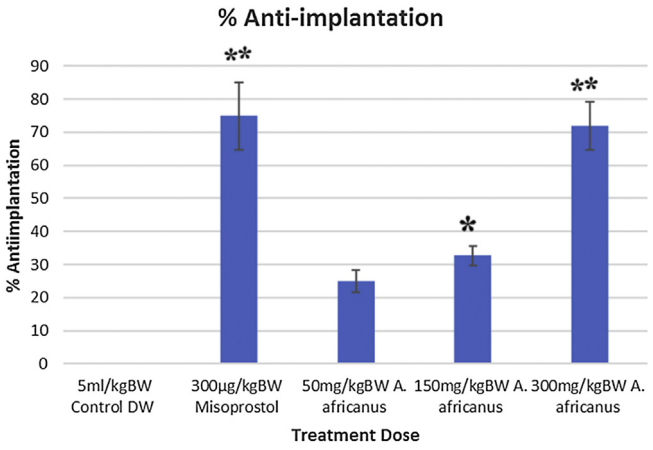

Fig. 2. Effect of *Asparagus africanus* on anti-implantation activity in SD rats. Values are expressed as mean ± SEM, (n = 6), $p^* < 0.05$, $^{**}p < 0.005$ vs. control. kgbw, kg per body weight; DW, distilled water.

For evaluation of anti-implantation (interceptive) activity, the results showed that the *A. africanus* crude extracts at doses 150 and 300 mg/kg bw significantly ($p < 0.05$) reduced the number of implantations and exhibited a higher anti-implantation activity as compared to untreated rats (Fig. 2). Findings also showed that there was significant ($p = 0.019$) increase in the percentage of anti-implantation in Misoprostol treated rats comparing to negative control, whereas there was no significant difference between Misoprostol and the highest dose 300 mg/kg bw of *A. africanus* treated groups. The extract did not cause premature delivery, post-implantation loss, however the highest dose of *A. africanus* exhibited 71.85% anti-implantation effect.

The photomicrographs of ovaries tissue obtained from treated female rats with different doses of *A. africanus* and control are shown in Fig. 3. Ovaries of rats in control group showed normal multiple follicles at different stages of development, while ovaries in misoprostol treated rats showed regressive corpora luteum and few empty follicles. Ovaries of rats treated with 50 mg/kg and 150 mg/kg of *A. africanus* demonstrated vacuolization and fatty acid deposition as compared to control, while ovaries of rats treated with 300 mg/kg of *A. africanus* showed regressive corpora luteum and decreased in oogenesis.

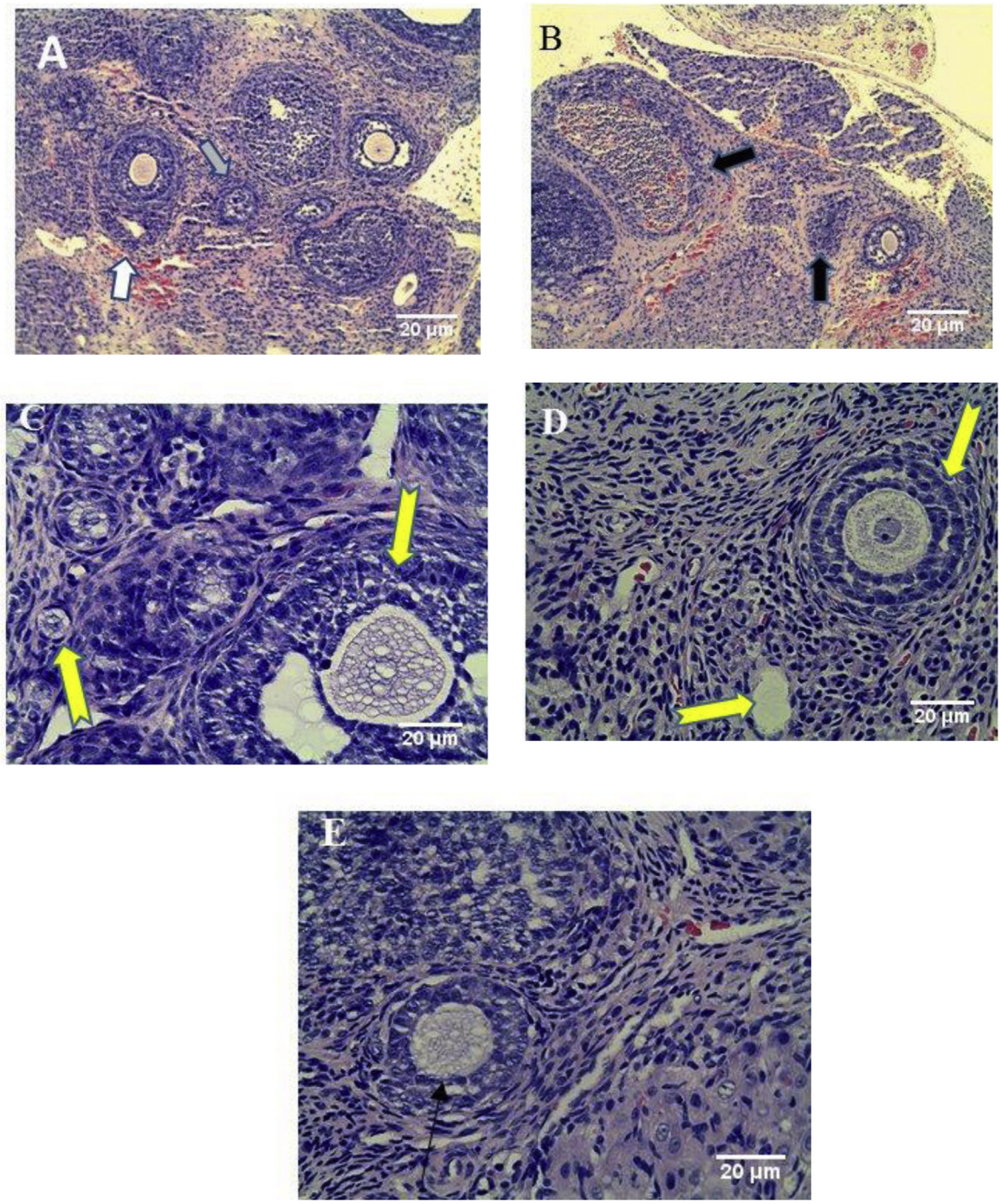

Fig. 3. Photomicrographs of ovaries from treated and untreated female rats, (magnification ×100).
(A) Normal control 5 mg/kg bw (dH_2O), showing small [⇩] and large [⇧] follicles, ovary with multiple follicles at different stages of development, ovulated follicle seen. (B) 300 µg/kg bw misoprostol treated group showing regressive corpora lutea [⬇]. (C) 50 mg/kg bw *Asparagus africanus*. (D) 150 mg/kg bw *A. africanus*, showing the histology of ovary, vacuolization and fatty acid deposition [⬆]; (E) 300 mg/kg bw *A. africanus*, showing regressive corpora lutea, section of ovary also showing decrease in oogenesis [↗].

LC/MS analysis and phytoconstituents identification

LC/MS chromatogram of aqueous extract of *A. africanus* is presented in Fig. 4. The mass spectral analysis has identified eleven compounds with some of them having steroidal nucleus such as asparasaponin II, sarsasapogenin, stigmasterol, and spirostan. Other non-steroidal compounds identified are acetylcaranine; 1,3,6,8-naphthalenetetrol; prosopinine; sugeonyl acetate; glutinosone; pandaroside C; and cinncassiol C3 (Table 1).

Acute oral toxicity of Asparagus africanus *extract*

The results of the effects of aqueous extract of *A. africanus* on body weight and organs weight, serum biochemistry and

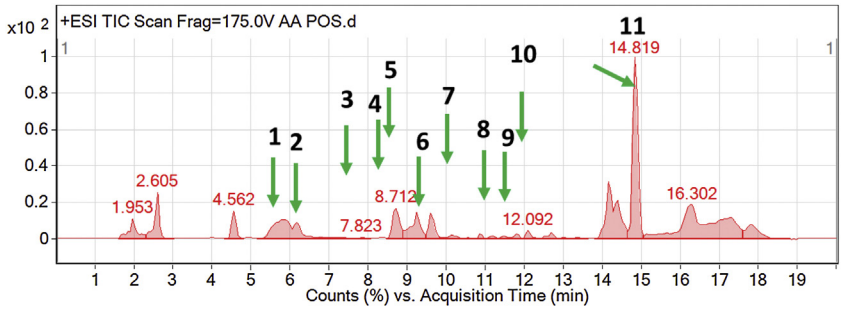

Fig. 4. LC/MS Chromatogram of aqueous extract of *Asparagus africanus*.

Table 1
Phytochemical compounds identified in *Asparagus africanus* aqueous extract using LC/MS Q-I-TOF.

Peak no.	RT (min)	Abund.	*m/z*	MW (g/mol)	MF	Tentative name
1	5.621	7399.91	314.13	313.13	$C_{18}H_{19}NO_4$	Acetylcaranine
2	6.183	149486.8	193.05	192.04	$C_{10}H_8O_4$	1,3,6,8-Naphthalenetetrol
3	7.414	852.11	1221.58	1198.59	$C_{56}H_{94}O_{27}$	3-*O*-(Xylb-3Glcb1-2(Xylb1-3)Glcb1-4Galb)-(25*R*)-5α-spirostan-3β-ol
4	8.279	1590.71	1089.54	1066.55	$C_{51}H_{86}O_{23}$	Stigmasterol
5	8.628	1466.3	903.49	902.48	$C_{45}H_{74}O_{18}$	Asparasaponin II
6	9.483	8122.24	887.49	886.49	$C_{45}H_{74}O_{17}$	Sarsasapogenin
7	10.01	7503.54	288.25	287.24	$C_{16}H_{33}NO_3$	Prosopinine
8	11.11	1196.92	299.16	276.17	$C_{17}H_{24}O_3$	Sugeonyl acetate
9	11.57	9217.11	143.13	220.14	$C_{14}H_{20}O_2$	Glutinosone
10	12.11	3153.01	763.42	740.43	$C_{39}H_{64}O_{13}$	Pandaroside C
11	14.83	2762850	383.20	382.19	$C_{20}H_{30}O_7$	Cinncassiol C3

Table 2
Effect of *Asparagus africanus* aqueous extract on the body and organs weights of treated and untreated rats.

Treatment dose	Initial body weight (g)	Final body weight (g)	Weight gain (%)	Liver weight (g)	Kidney weight (g)
5 ml/kg bw DW control	188.23 ± 11.33	268.40 ± 18.17	42.59	10.23 ± 0.18	2.76 ± 0.08
5 mg/kg bw *A. africanus*	197.32 ± 18.96	286.38 ± 19.76	49.66	10.51 ± 0.41	2.59 ± 0.08
50 mg/kg bw *A. africanus*	199.93 ± 10.01	253.60 ± 5.35	26.84	11.06 ± 0.11	2.93 ± 0.04
300 mg/kg bw *A. africanus*	215.18 ± 0.87	284.52 ± 7.93	32.22	12.14 ± 0.21	2.50 ± 0.06
2000 mg/kg bw *A. africanus*	229.24 ± 7.25	256.01 ± 8.15	11.68[a]	**13.21 ± 0.31**	**2.54 ± 0.11**

Values are expressed as mean ± SEM, n = 3 for each group.
[a] $p < 0.05$ vs. control group.

Table 3
Effect of *Asparagus africanus* aqueous extract on biochemical indices of treated and untreated rats.

Treatment dose	ALT (U/l)	ALP (U/l)	AST (U/L)	Cr (μmol/l)	Urea (mmol/l)	Uric acid (μmol/l)	Total protein (g/l)
5 ml/kg bw DW control	56.00 ± 10.23	230.00 ± 24.17	160.00 ± 27.07	22.33 ± 0.94	5.73 ± 0.59	72.33 ± 4.92	55.33 ± 4.92
5 mg/kg bw *A. africanus*	66.33 ± 7.72	172.33 ± 132.39	199.00 ± 68.68	21.33 ± 2.05	4.57 ± 0.69	102.33 ± 57.19	53.67 ± 1.25
50 mg/kg bw *A. africanus*	62.33 ± 2.49	201.00 ± 21.97	160.67 ± 30.03	22.33 ± 2.36	4.63 ± 0.34	67.67 ± 10.34	56.33 ± 2.49
300 mg/kg bw *A. africanus*	58.67 ± 8.58	168.33 ± 23.61	191.67 ± 50.16	24.33 ± 0.47	4.47 ± 0.66	92.33 ± 50.00	59.33 ± 3.09
2000 mg/kg bw *A. africanus*	55.0 ± 8.52	182.67 ± 6.18	141.33 ± 4.99	23.00 ± 0.82	4.90 ± 0.54	56.33 ± 10.40	56.67 ± 2.62

ALT, alanine aminotransaminase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Cr, creatinine.
Values represent mean ± SEM, n = 3. One-way ANOVA was used to analyze the data and $p < 0.05$ was considered as statistically significant.

Table 4
Effect of *Asparagus africanus* aqueous extract on haematological parameters of treated and untreated rats.

Treatment dose	HGB (g/l)	HCT (l/l)	RBC (10^{12}/l)	MCV (fl)	MCH (pg)	MCHC (g/l)	RDW (%)	WBC (10^9/l)	Platelet (10^9/l)
5 ml/kg bw DW control	135.67 ± 1.70	0.43 ± 0.00	6.84 ± 0.05	63.33 ± 0.47	19.4 ± 0.65	314.67 ± 2.49	11.90 ± 0.50	9.33 ± 1.25	1066.33 ± 65.19
5 mg/kg bw *A. africanus*	135.67 ± 11.12	0.46 ± 0.03	7.40 ± 0.51	60.00 ± 0.82	18.60 ± 0.33	308.67 ± 4.11	12.23 ± 0.82	11.50 ± 1.73	1098.67 ± 58.54
50 mg/kg bw *A. africanus*	138.67 ± 8.50	0.45 ± 0.02	7.34 ± 0.41	61.00 ± 1.63	18.90 ± 0.33	285.00 ± 39.63	11.33 ± 0.33	11.93 ± 5.36	1128.33 ± 23.16
300 mg/kg bw *A. africanus*	146.67 ± 1.70	0.47 ± 0.00	7.49 ± 0.23	62.67 ± 1.70	19.60 ± 0.42	313.00 ± 1.63	11.30 ± 0.29	12.93 ± 1.78	1094.33 ± 60.81
2000 mg/kg bw *A. africanus*	136.00 ± 1.6	0.43 ± 0.02	7.34 ± 0.05	60.33 ± 0.94	18.53 ± 0.17	307.33 ± 0.94	11.37 ± 0.12	7.83 ± 0.21	1022.33 ± 92.47

RBC, red blood cell; WBC, white blood cell; PCV, pack cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; HCT, haematocrit; RDW, red cell distribution width; PLT, platelet count.
Values are expressed as mean ± SEM, n = 3. One-way ANOVA was used to analyze the data.

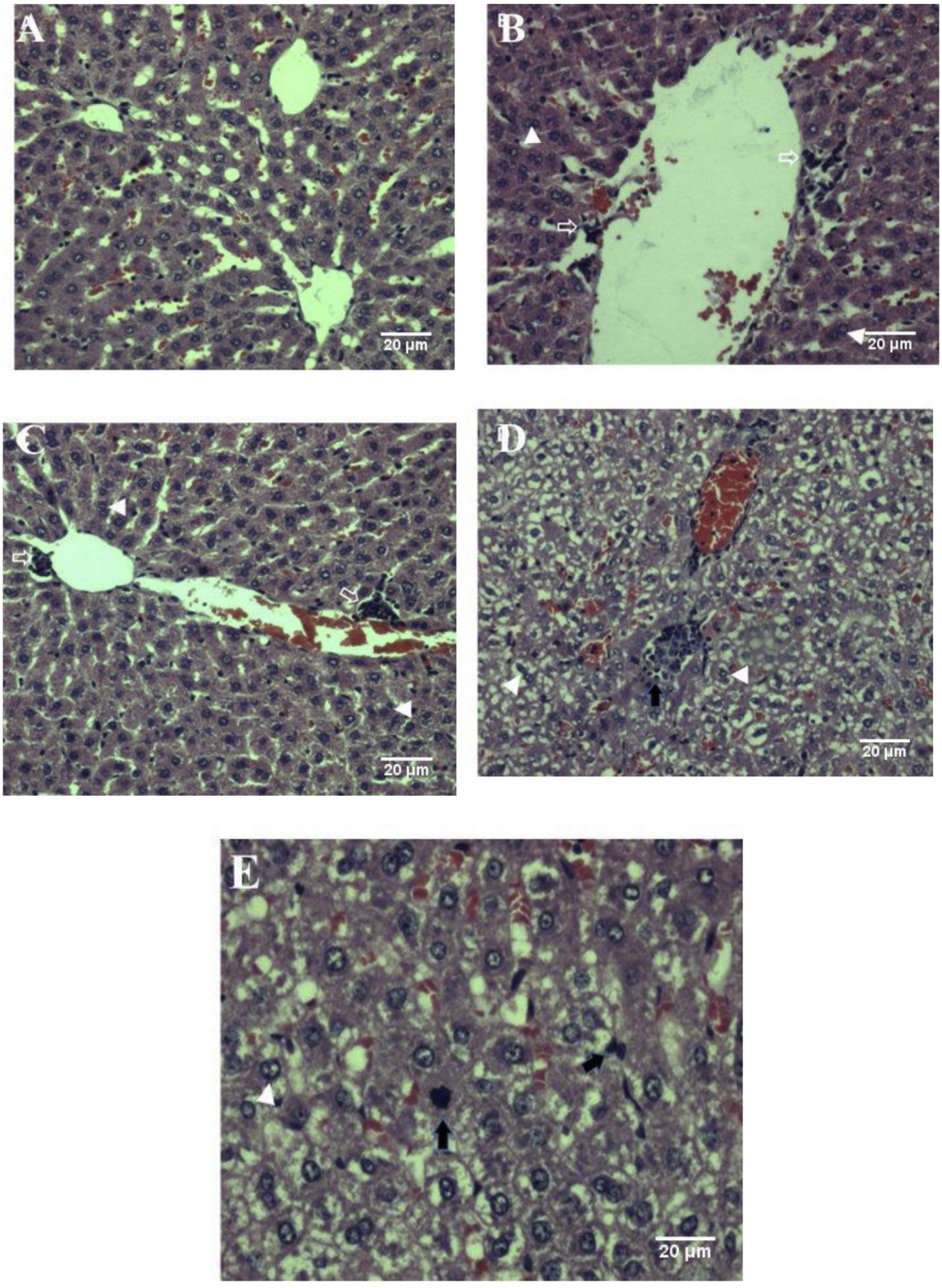

Fig. 5. Photomicrographs of liver sections in rats treated with *Asparagus africanus* aqueous extract. (H&E stain, magnification ×100). (A) Control: no bile stasis, central veins normal, portal tract unremarkable. (B) 5 mg/kg bw *A. africanus* and (C) 50 mg/kg bw of *A. africanus:* Liver in (B) and (C) shows occasional hepatocyte with binucleation [◁], but no bile stasis or steatosis. Perivenous inflammation [⇨] and lytic necrosis was noted in other areas. Portal tracts are unremarkable. (D) 300 mg/kg bw and (E) 2000 mg/kg bw of *A. africanus*: Liver in (D) and (E) shows moderately swollen (abundant clear cytoplasm) and binucleated hepatocytes [◁]. Perivenous inflammation, lobular hepatitis [⬆] and lytic necrosis noted.

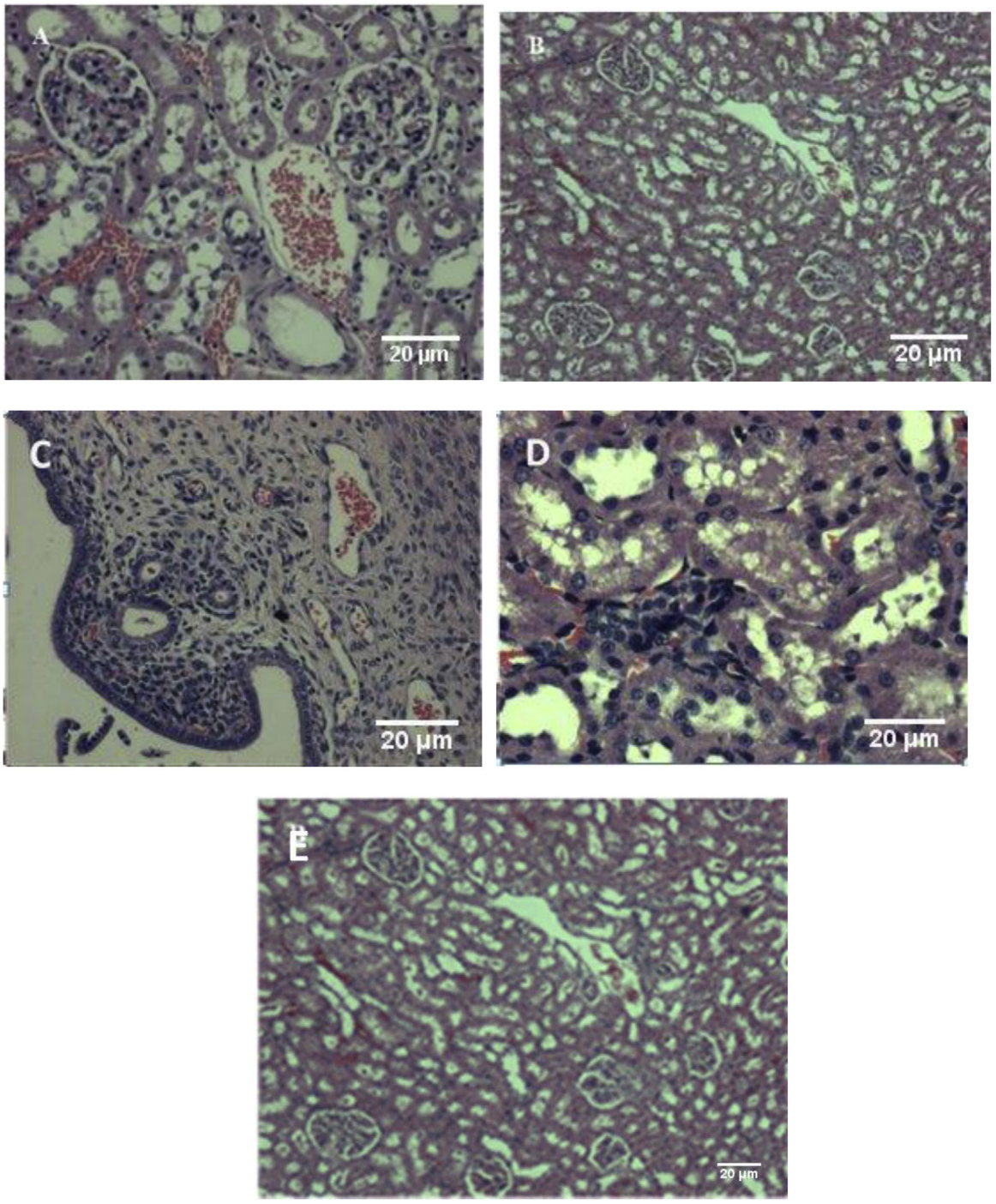

Fig. 6. Photomicrographs of kidney sections in rats treated with *Asparagus africanus* aqueous extract showed kidneys with normal glomeruli, tubules and blood vessels (H&E stain). (A) Control (magnification ×100). (B) 5 mg/kg bw *A. africanus* (magnification ×40). (C) 50 mg/kg bw of *A. africanus* (magnification ×100). (D) 300 mg/kg bw of *A. africanus* (magnification ×200). (E) 2000 mg/kg bw of *A. africanus* treated rat (magnification ×40).

haematology are summarized in Tables 2–4. Figs. 5 and 6 show photomicrographs of the liver and kidneys of treated and untreated rats with aqueous extract of *A. africanus*.

Body weight gain and organs weight

The mean body weight gain of all treated rats, except 5 mg/kg bw group, were less than the mean weight gain of rats in control group. The average weight gain in 2000 mg/kg bw group was approximately 3.6 times lower than that gain in control group (Table 2). Likewise, for 50 mg/kg bw group was also 1.5 times lower than the control group. However, the difference in the percentage of weight gain in the control and the treated groups were not statistically significant compared to control group, except for the highest dose of 2000 mg/kg bw ($p < 0.032$). Liver and kidneys mean weights showed no significant difference between treated and untreated rats (Table 2).

Effect of *Asparagus africanus* aqueous extract on biochemical and haematological parameters

Liver function and kidney function biochemical parameters (ALT, ALP, AST, Cr, urea, uric acid and total protein) were not significantly different between treated and control groups (Table 3). In addition, Table 4 shows the effects of oral administration of the aqueous extract of *A. africanus* on the haematology parameters compared to control group. The values for HGB, HCT, RBC, MCV, MCH, MCHC, RDW, WBC and platelet between control and treated groups were all not statistically significant.

Histopathology

Liver sections obtained from the rats of control group showed no bile stasis, normal central veins and portal tracts. On the other hand, 5, 50, 300 and 2000 mg/kg bw treated rats showed congested, moderately swollen hepatocytes with binucleation (Fig. 5A–E). Although no bile stasis was seen in all treated groups, perivenous inflammation, and lytic necrosis were observed (Fig. 5C and D). The portal tracts were also not remarkable. For kidney samples, both control and the highest dose group (2000 mg/kg bw) have normal glomeruli, tubules, interstitium and blood vessels (Fig. 5A and B).

Discussion

Asparagus africanus root extract was evaluated for anti-implantation activity. Interestingly, the high dose of *A. africanus* (300 mg/kg bw) showed significant increase in anti-implantation activity (71.85%) as compared to untreated rats and this indicates a potential interceptive activity of the root extract of *A. africanus*. For implantation of zygote, it is important that oestrogen and progesterone are in equilibrium and any disturbance in the levels of these hormones can cause failure in implantation activity (Geun-Shik et al., 2005). Our unpublished data demonstrated that the extract produced a dose-dependent increase in the oestrogen levels as compared to untreated rats. The oestrogenic properties of *A. africanus* could explain the anti-implantation properties of this plant. On the other hand, antagonizing the action of progesterone will result in the down-regulation of progesterone-dependent genes, with decidual necrosis and detachment of the products of conception.

More closely, to our result is the report by Geremew et al. (2006) on the antifertility of *A. africanus* using ethanol, and aqueous extracts. Their results further explained the possible hormonal changes as a result of the treatment with this plant, which may be responsible for certain reproductive abnormalities in experimental rats.

Histopathology evaluations were conducted on ovary tissues according to Theise (2007). Results revealed that the ovaries of rats in negative control showed normal, small and large follicles. In addition, ovary with multiple follicles at different stages of development, and old ovulated follicles were also reported. For positive control misoprostol treatment group, ovary with regressive corpora luteum and empty follicles were clearly seen. Histology of ovaries of rats treated with 50 and 150 mg/kg of *A. africanus* showed vacuolization and fatty acid deposition. Meanwhile, in 300 mg/kg bw treated rats there were regressive corpora luteum and decreased in oogenesis. However, one of the limitation of this study is that the quantification of follicles and corpora lutea were not reported and instead qualitative histological examinations were carried out.

Several studies have reported that *A. africanus* plant has potent anti-fertility properties which initiated their pre-natal and post-natal applications among women (Geremew et al., 2006). This study screened the bioactive phytochemicals responsible for those pharmacological properties of *A. africanus* plant. The presence of many phytochemicals can be linked to different pharmacological activities exhibited by plant extract. Aqueous extract was used in this study because it is often and the most available solvent used in traditional medicine preparations. In our study, the percentage of yield extract obtained from aqueous extract of the roots of *A. africanus* was 7%, and this is comparable to that reported by Madikizela (2014) which resulted in 6.8%. On the other hand, this amount of yield extract is more appreciable when compared to that reported by Moshi et al. (2003), which yielded 1–2% only of starting material of *A. africanus*. In another study, a modified traditional crude saponin extraction was used and the yield was 3.59% extract from the root of *A. africanus* Asfaw et al. (1999). Methods of extraction, nature of soil, topography and geographical location are some of the factors that might be responsible for these differences.

The present finding from LC/MS analysis indicated the presence of eleven phytochemical compounds; of which only four of them have a cyclic steroidal nucleus; namely asparasaponin II, sarsasapogenin, stigmasterol, and spirostan. The current result further confirmed and in line with the implicated steroidal saponins reported by Asfaw et al. (1999). These steroidal saponins might have different pharmacological and physiological effects on pregnant rats or might act synergistically to produce the oestrogenic effects of *A. africanus*. Other non-steroidal compounds identified are: 1,3,6,8-naphthalenetetrol, prosopinine, sugeonyl acetate, glutinosone, pandaroside C, and cinncassiol C3.

The presence of flavonoids is similar to that reported by Charles and Robert (2004) in *Trifolium pratense* known as red clover which shown to contain isoflavones and their effects are similar to that of diethylstilbestrol (Charles and Robert, 2004). Unfortunately, the undesirable effect of red clover on sheep fertility endangered the New Zealand economy in the past (Charles and Robert, 2004). Sapogenin and furostanol also have been isolated from *A. africanus* which shown an antiprotozoal activity (Oketch-Rabah et al., 1997). The reported sapogenin is in line with our study where asparasaponin II and sarsasapogenin were also reported in *A. africanus* using LC/MS method of analysis.

Findings of this study confirm that *A. africanus* contains some potential steroidal like compounds, which could explain the traditional uses of the root part of this plant as contraceptive. The antifertility activities of other species of *Asparagus* has been documented with the suggestion that the root part of *A. pubescens* has anti-implantation or direct effect on the uterus (Nwafor et al., 1998). Ethnobotanical studies of 152 medicinal plants of Madagascar used by local people have also revealed eight native species that are widely used by women for treating placental apposition and complications during childbirth. Moreover, it has also been used for the treatment of tropical illnesses such as malaria, filariasis, and sexually transmitted diseases like gonorrhea and syphilis. Among eight widely used plants by women in the region of Madagascar, Asparagaceae was particularly used for birth related properties (Razafindraibe et al., 2013).

Investigation of the acute toxicity is often the initial stage in almost all screening of unknown potential medicinal herbal substances. Most acute toxicity data are used to predict the safe dose limit in clinical application because some herbs exert toxic effects even at a very low dose. Hence, multiple doses study is immensely valuable in evaluating the safety profile of medicinal plants (Woode and Abotsi, 2011; Abiodun et al., 2015).

In current study, acute toxicity of root extract of *A. africanus* was administered at doses of 5, 50, 300 and 2000 mg/kg bw. At all dosed groups, there were no abnormal changes in the general appearance and behavioural characteristics of animals and no mortality was also recorded for all administered doses.

A study by Kebede et al. (2016) has been reported that the butanol extract of *A. africanus* showed no toxicity at a tested

concentration in mice. However, focal lobular inflammation of liver was noticed in few mice, hence this was attributed to the presence of flavonoid glycoside in the butanol fraction of the extract. They reported that *A. africanus* at 300 and 600 mg/kg bw has no hepatonephrotoxic and heamatotoxic effects (Kebede et al., 2016).

The mean percentage of weight gain for aqueous extract of the control group was 42.59%, which is higher than the mean weight gain of rats in 50, 300 and 2000 mg/kg bw treated groups. However, these reductions in weight gain in treated groups were not significantly different except for 2000 mg/kg bw ($p < 0.032$) when compared with that of control group. The biochemistry markers of the liver and kidney function tests showed no significant difference between treated and untreated rats.

Meanwhile, HGB, RBC, RDW, HCT, MCV and MCH were not statistically significant between treated and control groups. In addition, histopathology was conducted on formalin-fixed paraffin-embedded experimental animal tissues according to Theise (2007). Results showed that liver and kidney appeared normal and showed no remarkable difference between control and treated rats. However, the liver in high doses (300 and 2000 mg/kg bw) treated rats showed congestions with mildly swollen hepatocytes and, occasional binucleation, but no bile stasis or steatosis. Ultimately, *A. africanus* root extract causes no death or abnormal behavioural effects in rats, however it has an adverse outcome on the implantation and number of implant in female rats.

Conclusion

Asparagus africanus aqueous root extract has anti-implantation effect in female SD rats. *A. africanus* root extract probably has interceptive effect mainly by inhibiting implantation and the possible mechanisms could be through the oestrogenic and antiprogestin effects. LC/MS analysis revealed the presence of four steroidal saponins spirostan, stigmasterol, asparasaponin II, and sarsasapogenin in the aqueous root extract of *A. africanus*. This result can be used to justify its various uses in traditional medicine especially in birth related applications. The acute toxicity study showed that *A. africanus* aqueous root extract is safe up to 2000 mg/kg bw. Further pharmacological studies should be carried out to investigate the estrogenic effect and the antifertility activities of this plant as well as its bioactive steroidal saponins.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures used in this research were in accordance with the regulations and approval of the ethics committee for animal experimentation, Faculty of Medicine, University of Malaya (Ethics Reference no.: 2015-180505/PHAR/AEI).

Authors' contributions

AE conceived the study, performed the experiment, animal health monitoring, and assisted in manuscript preparation. ZC and MAA designed and coordinated the study. KM performed pathology examination. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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