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Research Article

Effect of Aqueous Extract of *Talinum triangulare* on Reproductive Parameters of Female Wistar Rats

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Abstract

Talinum triangulare commonly called water leaf, grows under humid temperature up to 10-15 cm in height, germinate in five days after planting with rapid growth pattern in presence of sufficient water and in 3 weeks becomes ready for harvest and consumption. There was positive effect of *T. triangulare* on the reproductive parameter of matured female Wister rats. Twelve groups of rats (5 each) were orally administered the extracts at three doses; 0, 30, 120 and 700 mg/kg/wt./ day for a period of 21 and 28 days. Body mass and vaginal smear were collected and recorded every seven days and every day except the pregnant Dams in the pregnancy outcome study groups had virginal smear collection discontinued when sperm was present in the virginal which was day zero of pregnancy, only animals on pro-estrus phase were either anesthetized or mated. Pregnancy outcome parameters such as leitter size, eye opening day, fur appearing day, were examined in pregnancy outcome study, other reproductive parameters such as leuthenizing hormones (LH), follicle stimulating hormones (FSH) and Estrogen-2 (E-2) hormones were observed. The findings revealed a significant increase in body mass in all groups administered with the extract when compared to the control, the extract groups were seen to have significant increase in weight of reproductive organs. Hormonal assays showed observable elevation of hormonal levels at the estrus phase on the following parameter: LH, E-2 and FSH. Hematological indices of dams were done using standard procedures. The study showed that the plant extract was safe at 30, 120 and 700 mg/kg. Therefore, *T. triangulare* leaves improved reproductive parameters and efficient in the management of pregnancy, infertility and as blood builder in children and adult.

Keywords: Pregnancy; Infertility; Oestrous Cycle; Inhibins; Leitter Size

Introduction

Talinum triangulare (water leaf) is classified in the kingdom: Plantae belonging to the kingdom: virridae plantae which is a phylum of Spermatophytina and of the order Caryophylare belonging to the family Portulacaceae with the common name water leaf [1]. It grows under humid temperature and grows up to 10-15 cm in height, its germination occurs five days after planting with very rapid growth pattern in presence of sufficient water supply and usually 3 weeks after cultivation it becomes ready for harvest. Nutritional studies on *T. traingulare* showed that the leaves contained per 100g edible portion thiamine 0.08 mg, niacin 0.3 mg, riboflavin 0.18 mg, ascorbic acid 31 mg, water 90.8 g, energy 105k (25 Kcal), protein 2.4g, fat 0.4g, carbohydrate 4.4g, fiber 1.0g and calcium 21 mg [2]. *T. triangulare* is useful in the treatment of diabetes, jaundice and other related diseases, useful in management conditions such as anemia, in pregnant women, growing children and adult. It is used as a natural source of blood booster [3]. Great deal of nutritional and health benefits has been derived from the use of T. triangulare and other medicinal herbs which are safer and with more therapeutic values than synthetic drugs, hence the study of more medicinal plants has become necessitated by increased scientific study on their bioactivity [4].

The increased occurrence of premature birth results in regular incidence of neonatal death and sickness, preterm labour refers to the commencement of labour before the expected delivery date resulting in the delivery of an under-developed newborn, this necessitate the quest for a perfect tocolytic agent that is known to uniformly effect absolute feto-maternal wellbeing. These types of agents vary in cost, utero-specificity, efficacy as well as safety [5]. The majorly known tocolytic agents include calcium channel blockers, beta-agonists and salbutamol an oxytotic receptor antagonist.

Materials and Methods

Sample collection

Fresh leaves of *T. triangulare* were obtained from University of Benin in the Faculty of Pharmacy, University of Benin, Benin City with voucher number UBH–T381. The leaves were dried at room temperature after collection then put in an oven at 40^0C for 2 days. The dried leaves were then Grinded into fine powdery form and filtered with 30- mesh sieve. The dehydrated sample of leaves was stored in an air tight container.

Figure 1: Photograph of the plant.

Extraction of plant materials

The powdered plant material was extracted by cold maceration with methanol for 24 hours, 3000 ml beaker and a cylinder of 2000 ml. The resulting crude extract was then filtered by passing through a cheese cloth followed by concentrating in a water bath, and drying in an oven at a controlled temperature of 40^0C for 48 hours (2 days).

Experimental animals

Female and male albino rats weighing approximately 180 g and 200 g were obtained from pharmacology animal house University of Benin, Benin City. These animals were also adapted and maintained in the animal house with standard laboratory conditions met and animals fed with pelletized feed and tap water *ad libitum.*

Drugs and chemicals

Drugs/chemicals procured in the course of experiment include: Sabultamol (GlaxoSmith Kline), oxytocin injection (laborate pharmaceutical Indian) and aqueous extract of *T. triangulare.*

Mating procedures

Female rats were mated in mating cages with their male canter pats in a ratio of 1:1 at pro-estrus. Successfully mated females were separated from the males, after microscopic examination for presence of sperm in the virginal and considered as day zero of pregnancy.

Pregnancy out comes

The Dams were divided into the various sub groups 1a - 6a and 1b - 6b treated with 0, 0.5 iu oxytocin intraperitoneally, 10 mg/kg salbutamol and 30,120, 700 mg/kg/bw. per-oral. The animals in sub groups (a) had uterine horns observed for implantation and resumption sites, was compared in requisites of litter size, while animals in sub group (b) were allowed to litter and the pups in this group were compared in terms of pup weight, mortality in 48 hours, day of appearance of furs and eye-opening day. Animals were observed daily for toxicity signs, diarrheal, salivation, jaw movements, writhing, convulsions, hair loss moribund or mortality, for period of 21 days, the dams were laparotomized under chloroform anaesthesia with sterile procedures. The uterus was observed, for the presence and location of resumption sites and for live and dead Leitters. The animals in sub-groups (b) that were left to give birth, parturition day were recorded. The number of live or dead pups was recorded, and their body weight was determined. Pups were examined for gross outer congenital abnormalities such as (clubfoot, anomalies of tail, open eyelids). Pup death rate up to 6 days (mortality), eye opening day, and fur appearance was recorded. Based on these data, the parameters were computed as follows : quanta pregnancy = (number of pregnant dams/number mated) x 100; implantation index = (total number of implants/number mated) x 100; pre-implantation loss = (number of corporalutea -number of implantations)/number of corporalutea) x 100; post-implantation loss =[(number of implantations - number of viable implantations)/number of implantations]x 100; viability index = (number of viable pups on day 4 after delivery/number of live born pups) x 100; birth index = (number of Pups born/number of implantations) x 100; foetal survival ratio = (number of surviving pups/number of implantations) x 100; live birth index = (number of live born pups/total number of pups born) x 100; gestation index= (number of live pups/numbers of pregnant dams) x 100.

And 100 mg/ml were also determined. The effect of sabultamol

Estrogenicity

The female animals on attainment of three regular consecutive oestrous cycles were divided randomly into four groups, (5) five rats each and treated as follows: control- received distilled water, 30, 120 and 700 mg/kg/day aqueous leaves extract of *T. triangulare.*

At the end of the 21day periods, the following parameters were evaluated:

Ovaries

- Appearance of ovary after 21 days of treatment,
- Nature of oocytes,
- Production of secondary follicles,
- Level of serum hormones of LH, FSH, E-2 cells.
- Reproductive organ weight in relation to body weight.

Statistical analysis

The outcome from the studies was articulated as the mean ± SEM (standard error of mean). Statistical analysis was carried out using graph pad prism 6 version software (UK). One-way analysis of variance (ANOVA), and comparisms between the control and treated groups were analyzed using Dunnett's multiple comparisms test. $P < 0.05$, 0.01, 0.001 and 0.0001 was regarded as indicating significant difference in all cases.

Results and Discussion

The table 1 below shows abortifacient effect of oxytocin, standard drug sabultamol and the pregnancy enhancing effect of different doses of extract on pregnancy outcome. The result shows that the extract was able to prevent preterm labor and abortion see (Table 1) below. Normal control, negative control which received oxytocin with high presence of resumption sites, positive control, 50, 150 and 500 mg/kg extract treated respectively.

The table shows effect of oxytocin, sabultamol and the pregnancy enhancing effect of different doses of T. triangulare on pregnancy outcome. The result indicates that the extract was able to prevent preterm labour and abortion see (Table 2) below. Normal control, negative control which received oxytocin, positive control, 30, 120 and 700 mg/kg extracts treated.

The gravid rats administered 50, 150 and 500g/kg/bw, daily commencing from day 0- 21 and day7-26. Data were represented as means ± SEM a= $P<0.05$, b = $P < 0.01$, c = $p< 0.001$ and 0.0001.

Parameter	Control (N=5)	Nc (N =5)	Pc (N=5)	30,120,700 mg/kg Mp (N=20)	30,120,700 mg/kg LP (N=20)
NuI	(7)	(0)	5	(6-10) (8-12) (10-12)	(5-10) (7-12) (10-12)
Qp (%)	100	0.00	100	100	100
Ii (%)	60	0	100	100	100
Pi (%)	54 ± 0.2	100 ± 0.1^{c}	26 ± 0.5^{a}	(80 ± 3.4$^{a)}$) (97 ± 0.2^{c}) (100 ± 0.2 ^{d})	(1.9^{a} ± 0.2) (1.3 ± 0.5) (1 ± 00)
GI (%)	100	100	100	100	100
Mgd	21.3 ± 0.2	21 ± 00	21.4 ± 0.3	(22.5 ± 0.3^{a}) (22.9 ± 0.1^{b}) (23.0)	(82.0 ± 0.6^{c}) (73.6 ± 0.3^{c}) (96 ± 01^{d})
LWS/g	3.0	2.8	3.5	4.0	5.2

Table 1: The effect of aqueous extract of *T. triangulare leaves* on leitter parameters of Dams.

Key; Qp: Quanta Pregnancy; Nui: Number of Uterine Implants; Mp: Mid Pregnancy; Nc: Negative Control; Pc: Positive Control; Mp: Mid-Pregnancy; Lp: Late Pregnancy; G1: Gestation Index; Mgd: Mean Gestation Days; LW/g: Leitter Weight on Sacrifice in Grams.

Parameters	Npb (g)	Nlbp (mean)	Pwb (g)	Number of Deformed Pups	Birth index (%)	Pup Survival Ratio (%)	Life birth Index (%)	Eye opening day	Afd
Control	5.8 ± 0.2	5.0 ± 0.1	4.0 ± 0.1	0.00	100	90.1 ± 0.4	100	19 ± 0.5	7.4 ± 0.3
NC	5.0 ± 0.4	5.0 ± 1.1	3.8 ± 0.3	0.00	100	33 ± 0.1	100	20 ± 0.3	9.1 ± 0.1
PC	4.9 ± 0.2	5.7 ± 1.0	4.7 ± 0.4	0.00	100	54.3 ± 0.1	100	20 ± 0.4	6.3 ± 0.3
30mg/kg	8.7 ± 0.5^{a}	7.0 ± 013^{a}	5.6 ± 0.1	0.00	100	96 ± 0.1^{b}	100	15.0 ± 0.2^{b}	7.0 ± 0.5^{b}
120mg/kg	9.88 ± 0.2^{c}	9.7 ± 0.3^{c}	6.09 ± 0.2^{a}	0.00	100	99 ± 0.4^{c}	100	14 ± 0.5^{c}	6.0 ± 0.4^{c}
700mg/kg	11.58 ± 0.3^{d}	12 ± 0.0^{d}	4.36 ± 0.4^{d}	0.00	100	100 ± 0.5^{d}	100	13.0 ± 0.5^{d}	6/0 ± 0.1^{d}

Table 2: Effect aqueous extract of *T. triangulare* leaves on litter parameters of female rats.

Key: Npb: Number of Pups Born; Nlbp: Number of Lives Born Pups; Pwb: Pup Weight at Birth; Ndp: Number of Deform Pup; Bi: Birth Index; psr: Pup Survival Ratio; Lbi: Life Birth Index; Eod: Eye Opening Day; Afd: Appearance of Fur Day; NC: Negative Control and PC: Positive Control.

Hematological investigation

The table below (Table 3) is a summary of the results obtained from hematological analysis of blood samples collected from the dams. The result reveals that the aqueous extract was found to have significantly increased white blood cell (WBC), Red blood cell (RBC), Pac cell volume (PCV), platelets (PLT), Neutrophil (NEU) and Basophil (BASO).

Body weight index

Results obtained from the body weight index of the dams during the course of pregnancy, revealed that even in the presence of induction of abortion the extract treated Dams had successful progression of pregnancy and development (Table 4).

Parameters	Npb (g)	Nlbp (mean)	Pwb (g)	Number of Deformed Pups	Birth index (%)	Pup Survival Ratio (%)	Life birth Index (%)	Eye opening day	Afd
Control	5.8 ± 0.2	5.0 ± 0.1	4.0 ± 0.1	0.00	100	90.1 ± 0.4	100	19 ± 0.5	7.4 ± 0.3
NC	5.0 ± 0.4	5.0 ± 1.1	3.8 ± 0.3	0.00	100	33 ± 0.1	100	20 ± 0.3	9.1 ± 0.1
PC	4.9 ± 0.2	5.7 ± 1.0	4.7 ± 0.4	0.00	100	54.3 ± 0.1	100	20 ± 0.4	6.3 ± 0.3
30mg/kg	8.7 ± 0.5[a]	7.0 ± 013[a]	5.6 ± 0.1	0.00	100	96 ± 0.1[b]	100	15.0 ± 0.2[b]	7.0 ± 0.5[b]
120mg/kg	9.88 ± 0.2[c]	9.7 ± 0.3[c]	6.09 ± 0.2[a]	0.00	100	99 ± 0.4[c]	100	14 ± 0.5[c]	6.0 ± 0.4[c]
700mg/kg	11.58 ± 0.3[d]	12 ± 0.0[d]	4.36 ± 0.4[d]	0.00	100	100 ± 0.5[d]	100	13.0 ± 0.5[d]	6/0 ± 0.1[d]

Table 2: Effect aqueous extract of *T. triangulare* leaves on litter parameters of female rats.

Key: Npb: Number of Pups Born; Nlbp: Number of Lives Born Pups; Pwb: Pup Weight at Birth; Ndp: Number of Deform Pup; Bi: Birth Index; psr: Pup Survival Ratio; Lbi: Life Birth Index; Eod: Eye Opening Day; Afd: Appearance of Fur Day; NC: Negative Control and PC: Positive Control.

Parameter	Wbc	Rb	Pcv	Plt	Neu	(Baso)
Control	14.0 ± 0.0	6 ± 0.1	35.0 ± 0.4	69.0 ± 2.2	17.0 ± 0.2	1.5 ± 0.1
30mg/kg	16.42 ± 0.2[a]	6.6 ± 0.1[a]	38.45 ± 0.1[b]	68.9 ± 0.3	20.98 ± 0.3[d]	1.040 ± 0.0
120mg/kg	17.3 ± 0.3[d]	6.10 ± 0.1[d]	47.84 ± 0.4[d]	69 ± 0.6	21.09 ± 0.2[d]	7.98 ± 0.2[d]
700mg/kg	18.00 ± 2[d]	6.0 ± 0.1[d]	64.82 ± 0.8[d]	69 ± 0.9	24.30 ± 0.3[d]	8.44 ± 0.2[d]

Table 3: Haematological analysis of blood samples from the dams.

Key: [a] p< (0.05), [b]p< (0.01),[c] p< (0.001) and [d] p< (0.0001).

Treatment	Day 0	Day 7	Day 14	Day 21
Control	180.35 ± 0.1	184.0 ± 0.8	184 ± 0.2	196 ± 0.1b
Nc	184.8 ± 02	186.7 ± 0.4	165 ± 0.2 [a]	147.1 ± 07 [d]
Pc	180 ± 0.1	184 ± 0.5[b]	185.1 ± 0.5	187 ± 0.2[c]
30mg/kg	188 ± 3.1	190.1 ± 3.9	193.4 ± 2.0[a]	249.6 ± 0.4[b]
120mg/kg	186 ± 1.0	202 ± 5.2 [a]	265 ± 4.1[d]	282.1 ± 2[d]
700mg/kg	183 ± 3.5	194.6 ± 5[a]	208 ± 2.6[c]	214.5 ± 1.2[d]

Table 4: Body weight index of the dams during pregnancy.

Key: [a] p< (0.05), [b] p (0.01) [c] p< (0.001), and [d] p< (0.0001).

Parameter	Control	30mg/kg	120mg/kg	700mg/kg
Body weight(g)	181.5 ± 0.3	187.7 ± 0.4[a]	194.04 ± 0.4[d]	2000 ± 0.1[d]
Reproductive organ weight(mg)	180[b] ± 0.2	217 ± 0.3[b]	218[c] ± 0.1	221 ± 0.4 [d]

Table 5: Body mass and reproductive organ system mass connected by means of daily administration of *T. triangulare* extract to mature female rats after 21 days treatment.

Key: [a] p< (0.05) [b] p (0.01) [c] p< (0.001), and [d] p< (0.0001).

Hormone	Control	30mg/kg	120mg/kg	700mg/kg
FSH (mlu/m)	4.2 ± 0.1	4.7[a] ± 0.1	4.69 ± 0.1	5.0[c] ± 0.2
EH (Pg/ml)	9.0 ± 0.1	13.0[b] ± 1.1	15.6[c] ± 1.2	9.0 ± 0.1

Table 6: Hormonal change connected with daily administration of *T. triangulare* to mature female rats.

Key: FSH = follicle stimulating hormone. LH: Values are mean ± SEM. n = 6, [a] P<0.05 in comparison with the control group. [b] P < 0.01. [c] P < 0.001. [d] P< 0.0001. Key Follicle stimulating hormone (mlu/m), Leuthenizing hormone (mlu/m) and Estrogen hormone Pg/ml

Histological findings

The photomicrograph below represents Rat ovary administered various doses of *T. triangulare* b (See figure 2). Plates A, B, C and D displays normal ovarian structural design composed of mature (oocytes, graphian follicles, stromal and secondary follicles,) in all treated groups.

Figure 2: Effects of *T. triangulare* (A) Control rat ovary composed of (a), secondary follicles and (b), stromal (H&E x 4), (B) Rat ovary administered with 30 mg/kg Extract showing (a), graphian follicles secondary follicle and (b), stroma (H&E x 40), (C) Rat ovary administered with 120 mg/kg Extract showing (a), secondary follicles and (b), stroma (H&E x 40), (D) Rat ovary dministered with 700mg/kg Extract showing (a), secondary follicle, (b), oocyte and, (c) stroma (H&E x 40).

Tocolytic effect of 50 mg/ml aqueous extract on spontaneous contraction of the uterus

Relaxant action of 30 mg/ml of *T. triangulare* on spontaneous contraction on the uterus, from the tocolytic assay, it was observed that the extract dose of 30 mg/kg extract inhibited spontaneous contraction on the uterus by 36.42% (See figure 3). Maximum induced relaxation values are the means of percentage responses ± SEM, n=5 experiment for each point with significant p-values.

Figure 3: Tocolytic effect of *T. triangulare* on the uterus.

Key: p< 0.01=b, p< 0.001=c, p< 0.001=d.

Tocolytic effect of 30 mg/kg/bw of aqueous extract on Oxytocin induced contraction on the uterus

The tocolytic effect of 30 mg/kg of aqueous extract on oxytocin induced contraction on the uterus, at a concentration of 6.4 x10-4 I.U/ml alone gave a mean response of 96.0% of maximum contraction, while the same concentration in the presence of the *T. triangulare* gave a mean response of 31.28% a clear case of uterine inhibitory effect with significant p-values (see figure 4).

Figure 4: The tocolytic effect of 30 mg/kg/bw aqueous extract on Oxytocin induced contraction on the uterus.

Values represent mean percentage responses ± S.E.M. (n = 4expreriments) for each point.

Key: p< 0.05=a, p<0.01=b, p< 0.001=c, p< 0.001=d.

Tocolytic effect of *T. triangulare* aqueous extract on acetylcholine induced contraction

At 6.4 x10^{-4} µ/ml of acetylcholine alone gave a mean response of 77.56% of maximum contractile response, while the same concentration in the presence of the plant extract gave a mean response of 39.08% contraction signifying inhibition at 30 mg/kg, (see figure 5).

Figure 5: Tocolytic effect of aqueous extract of *T. triangulare* on acetylcholine induced contraction on the uterus.

Key: p< 0.05=a, p<0.01=b, p< 0.001=c, p< 0.001=d values are indicated as percentage response ± S.E.M. n = 4 experiments E stands for aqueous extract, ACH stands for acetylcholine and S. stands for salbutamol.

Hematological results exposed that at the various doses of aqueous extract of *T. triangulare* there was observable increase in the counts of white blood cells (WBC), red blood cell counts (RBC) and Pac cell volume (PCV), lymphocyte counts LYM, Eosinophil (EOS), Basophil (BOS) and Platelet aggregation (plt) was found to have elicited increase levels of platelet aggregation in a dose dependent manner the increase in count of these cells of the haemagenic series tells that there may have caused activation of the regulation of the immune system resulting in the increase [3]. The increase of WBC counts explains that platelet (PLT) count was increased also, as the augmented counts of white blood cells (WBC) was likely to have obliged in boosting the immune system [6], the RBC cells and pack-cell volume (PCV) is known to enhance the immune system, the assessments of haematological parameters can be used in determine the extent of decree On RBC cells count was providentially enhanced as the dose was increased a sign that the extract could be a good source of tonic, yet the (PCV) was improved as this suggestive of poly-cythemia and positive erythopoisis [7]. The significant increase in RBC with a shift in PCV thus *T. triangulare* on animal's showed that it had no toxic effect on RBC. Because of the encouraging effect of the plant, PLT aggregation was also increased, LYM counts was also seen to increase, eosinophil EOS counts was enhanced and the counts of basophil BOS was greater than that of the control, This also supports the histological findings since the results remarkably suggest that *T. triangulare* have possible effect as a naturally derived immune tonic as a result of the immune boosting effects induced by *T. triangulare* [8]. Oral administration of *T. triangulare* to pregnant female rats resulted in weight gains, despite oxytocin administration at single dose level on day 12 of pregnancy informative that the extract prevented abortion and preterm labour [9,10]. Weight loss was observed in the negative control group administered oxytocin at single dose level signifying abortion and preterm labour. Over the 21 days of the conducted test, body weight of negative control group was seen much -less than normal control and extract treated group [11]. In disparity, the Dams administered *T. triangulare.* The body mass was progressively increased in all the groups in a dose dependent pattern, indicating that regardless of the induction of abortion with oxytocin and preterm labour on day 12 and 21 of pregnancy there was progressive leitter development associated with various doses of *T. triangulare* which was an indication of a positive pregnancy outcome on the dams, their leitters and pups [12,13]. Photomicrograph of rats' ovary in (Figure 1: B-D) was dose dependent with observable increase in growth of ovarian cells giving rise to appearance of graphian follicle, stroma, oocytes and secondary follicular cells which means that the extract suggestively caused increase female fertility level. The multipurpose use of *T. triangulare* in the management of medical various conditions by herbal practitioners in rural inhabitants were indicative of increased fertility level. The boost in body and reproductive organs mass may be due to the information that *T. triangulare* is a good supply of tonic that stir up appetite [3], encourage body mass gain and acts as carminative. *T. triangulare* is suspected to `possess hypoglycemic activity [2], given that hypoglycemia is amongst the stimulators of growth hormone (GH) secretion from the anterior pituitary gland [6]. The hormonal profile study showed increase in E2 cells which indicates that *T. triangulare* may possess some phytogenic property which may exist actively in little or much absorption and may affect other target tissues and acts as agonist or antagonist to the E2 hormones. These phyto-estrogens may be capable of manipulating basic cell biology to their effect on cellular enzymes. The increased level in serum LH after oral administration of *T. triangulare* may be attributed to elevated plasma concentrations of oestrogen for 1 to 2 days, as occur during the oestrogen peak of the late follicular phase, which acts upon the pituitary to enhance the sensitivity of LH releasing mech-

anism to GnRH [7].The increased oestrogen level may stimulate further enhancement in secretion of GnRH by the hypothalamus based on earlier findings of [14]. Furthermore, pre- ovulatory secretion of progesterone, though limited, may result in a positive feedback on the oestrogen – primed pituitary to enhance LH release. The study resulted in decrease in serum level of FSH in oestrous phase of the oestrous cycle; this seemed not to affect ovulatory process. This can be explained as in the late follicular phase, FSH level decrease in relation to the rise in E2 levels which means that there is no need to increase FSH level in the late follicular or ovulatory phase. At the same time, it has been noticed that the dominant follicle survival (≥ 10 mm) is mainly LH – dependent [8]. The negative feedback of E2 is predominantly exerted on FSH secretion at the pituitary level. Just before ovulation, after the GCs acquire LH receptors, LH also stimulate the production of inhibin by these cells. The inhibins inhibit FSH production by the gonadotrophs [15]. After ovulation, the CL secretes E2, progesterone and inhibin A under the control of LH [16]. The oestrogens exert negative feedback at both low and high concentrations, while progestins are effective only at high concentration. These vivid events lead to decrease in FSH level. Pregnancy outcome showed that *T. triangulare* may gainfully handle pregnancy problems like miscarriages and preterm birth which is the leading cause of birth of underdeveloped infants, in females with the problem of regular miscarriages of pregnancy before the completion of first trimester, *T. triangulare* may be helpful in preventing miscarriage as the negative control dams in table 1 was seen to possess high presence of resorption site. The leitter size seen in uterine horns of the dams treated with *T. triangulare* was more in number and grossly healthier when compared to the weight of Leitters in normal control and those of the positive control (Table 2), yet in the presence of induced abortion and preterm labor on day 12, on sacrifice at day 21 dams treated with *T. triangulare* had high presence of leitters up -to ten per a dam [17]. the results obtained from Dams in the subgroup (b) left to leitter, orally dams administered *T. triangulare* from day zero of pregnancy to twenty-one and at day twenty-one, oxytocin was given an hour after oral administration of various doses of the aqueous extract. It was observed that onset of labour was delayed for up to 73.3 hours. As a genuine proof that the plant extract may have caused the delay in onset of labour. The pups delivered by the Dams treated with the different doses of extract was observed to be grossly better in size, appearance and more in number and higher in weight [18].The death rate of the pups from normal control, negative control and the positive control was very high, as there was almost no survival recorded in 24 hours after their delivery. Meanwhile, for the extract treated dams there was a very high survival rates as there was almost no death recorded [6]. Oral administration of the *T. triangulare* during pregnancy had positive effects on Leitters in terms of uterine implants, gestation index/implantation index. On the other hand, it posed no substantial threat to successful pregnancy considered by the increase in pups survival ratio and birth index including reduction of post-implantation Losses [19]. These are seen as positive outcome in pregnancy giving rise to reduced mortality rates as evident at birth and laparotomy probably at mid stage of prenatal development since *T. triangulare* was proven non-toxic with no correlation existing between maternal toxicity and developmental toxicity, the use of the extract becomes accepted especially for women suffering incessant miscarriage and premature labour and are at high risk of miscarriage. The pups born by dams treated with the *T. triangulare* at 30, 120 and 700 mg/kg had high mean birth weights. This indicates intrauterine growth promotion were not due to increase of gestational length without incidence of preterm delivery. However, it could be attributed to increased blood flow through the placental and essential supply of nutrients to the pups. The viability index of pups born following foetal exposure to the leaves extract was high, even if some parameters of postnatal development like day of eye opening and day of appearance of fur remained unaffected. If these data can be applied to women, then consumption of the leaves extract during pregnancy can be encouraged in countries like Nigeria where more than one third of all infants born are small for gestational age. Normal birth weight has a major influence on neonatal wellbeing with less fear and occurrence morbidity. The result is consistent with the belief of traditional practitioners that the plant is used in the treatment of preterm birth and prevention of abortion with positive effects on litters which reveals enhancement of pup development and wellbeing. The uterotonic assay revealed that the aqueous extract was found to have relaxant activity on the uterus, as *T. triangulare* extract inhibited spontaneous contraction by 76%, acetylcholine and oxytocin contraction on the uterus was inhibited. *T. triangulare* might have been acting as a physiologic antagonist, meaning that it is likely for oxytocin to cause contraction as the extract is causing relaxation [20]. Assessments of the data revealed, that there was a significant inhibition in spontaneous contraction, oxytocin and acetylcholine contraction which were antagonized by the aqueous leaf extract at 30 mg/kg by contraction of 31.28% compared to oxytocin induced contraction at 96% and 39.08 % compared to acetylcholine induced contraction on the uterus at 77.56% with p-values at p-<0.01, p-< 0.001 and 0.0001 respectively (figures 4-5). The result in figure 4-5, showed that a reasonable inhibitory activity was induced by the plant extract [21]. On contraction induced by both oxytocin and acetylcholine on

calculation of the percentage difference in induced contraction of both oxytocin and alone in presence of extract, it was found that *T. triangulare* influenced a percentage inhibitory effect of 64.72% and 38.52% at 30 mg/kg of the extract with significant p-value of P< 0.01, p-<0.001 and p-<0.0001. On the acetylcholine induced contraction, since the maximum response was not obtained, it may specify that the extract was acting as non-competitive and nonspecific receptor antagonist. From this observation, it could be suggested that the mechanism of action of the plant might be through a non-specific receptor antagonism path way [22], thus *T. triangulare* elicited inhibition of spontaneous contraction on the uterus when compared to the intrinsic contraction of 100% to 36.42% when the percentage difference was calculated it was seen that the extract had inhibited spontaneous contraction by 76%.This results reveals that the aqueous extract of *T. triangulare* is a prospective uterine relaxant agent that may be possibly useful for alternative management or treatment for threatened abortion [23].

Conclusion

The present research has shown that *T. triangulare* has positive effects on fertility parameters with positive pregnancy outcome and blood building potentials of the aqueous extract.

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