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Effect of *Polyalthia Longifolia* Leaf Extract on Uterine Function Indices of Female Wistar Rats

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Abstract

The bark of Polyalthia longifolia plant is commonly used in the treatment of various diseases like diabetes, malaria and many others. In this research, phytochemical screening and effects of the aqueous extract of P. longifolia leaves on the uterine function indices of female Wistar rats were investigated. Phytochemical analysis of the extract was performed using standard methods. The animals were randomized into four groups (Control A, B, C and D) of five rats each. Animals in the control group were given 1ml of distilled water daily, for 30days. While those in groups B, C and D were administered with the same volume of 50, 100 and 200mg/kg body weight of the plant extract, respectively, for 30days as well. At the end of the administration period, the animals were sacrificed 24hours after the last day of administration. Uterine function indices were evaluated afterwards. Phytochemical screening indicated the presence of saponins, flavonoids, tannins, phenolics, cardiac glycosides, steroids,

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anthraquinones, phlobatannins and alkaloids while terpenoids were not detected. The extract at a moderate dose of 100mg/kg body weight gave a significant increase and decrease (P<0.05) in uterine glucose and uterine cholesterol respectively; it also revealed a significant increase and decrease(P<0.05) in alkaline phosphatase activity and total protein, when compared with the control. In general, it could be inferred that the aqueous extract of P. longifolia leaves possesses fertility enhancing potentials with regards to the uterus and uterine function indices.

Keywords: Phytochemical Screening, *Polyalthia longifolia*, Secondary metabolites, Uterine function Indices

Introduction

The utilization of traditional medicine in the cure of diseases is as old as man. Despite the improvement made by man in the production of pharmaceutical drugs, the decreasing effectiveness of synthetic drugs and the increasing contraindications of their usage, make the continued use of plants as important sources of medicinal agents (Petrovska *et al*., 2012). Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses (WHO, 2005). *P. longifolia* var. pendula of the (Annonaceae) family are mostly found in the tropical regions. Various parts of the plant have been used in traditional system of medicine for the treatment of fever, skin diseases, diabetes, hypertension and helminthiasis (Katkar *et al*., 2010). Several bioactive compounds are found in the leaves, stem and stem bark of the plant such as aporphine and azafluorene alkaloids (Wu *et al*., 1990), clerodane-type diterpenoids sesquiterpene compounds (Matharanda-Murpy *et al*., 2005) proanthocyanidins (Nairand Chanda, 2006). The genus *Polyalthia* belongs to the *Annonaceae* family (Chatrou *et al*., 2012). It is a type of flowering plant found in tropical and subtropical regions, including South Asia, South East Asia, and Australia (Chatrou *et al*., 2012). In India, the *Polyalthia longifolia* is also called Ashoka or Indian mast tree due to its special appearance as a Stupa and it is called 'Masquerade Tree' in Nigeria (Li *et al*., 2009; Folashade *et al*.,2018). Scientific reports on leaves, bark, stem bark, root, twigs, and seeds of *Polyalthia* have revealed dozens of types of alkaloids and terpenes with numerous biological and pharmacological activities with chemo-preventive potentials, such as anti-bacterial (Faizi *et al*., 2003; Marthanda Murthy *et al*., 2005; anti-viral (Kanokmedhakul *et al*., 2007); anti-plasmodial (Misra *et al*., 2010); anti-fungal (Bhattacharya *et al*., 2015); anti-inflammatory (Bermejo *et al*., 2019) ; anti-cancer effects (Yao *et al*., 2019) and anti-ulcer (Duan *et al*., 2020). The aim of this research was to evaluate the efficacy of aqueous extract of *Polyalthia longifolia* leaves on the uterine function indices of female Wistar rats.

Figure 1: Picture of mature *Polyalthia longifolia* leaves

Materials Methods

Materials

Collection and Authentication of *Polyalthia longifolia* leaf

Polyalthia longifolia leaves were obtained from a garden at Graceland Tanke, Ilorin, Kwara state. The leaves were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara state. A voucher specimen (with No. UILH/001/872), was deposited.

Experimental animals

Twenty female Wistar rats with an average weight of 124.90 ±2.31 g was acquired from the Animal housing unit of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Kwara State Nigeria. All the animals were housed in well-ventilated cages under conditions of temperature between 23-27 °C, photoperiod of 12hours natural light and dark cycle, relative humidity 45-55%.

Chemicals and assay kits

Assay kits used for the analysis of uterine glucose, total protein and cholesterol were supplied by Fortress Diagnostics Limited, Antrim technology park, Antrim, United Kingdom.

Methods

Preparation of aqueous extract of *Polyalthia longifolia* leaves

The plant samples were detached from the stem, washed under running tap water, oven dried (Uniscope, SM-9053, Surifriend medicals, England) and pulverized using an electric blender (Kenwood). A known mass (300g) of the grounded powder was then dissolved in 3000ml of distilled water for 48hours with intermittent stirring. The solution was sieved using a muslin cloth and then filtered using Whatman No. 1 filter paper. The filtrate was concentrated using a water-bath (SM- 8B, Microfield, England) at 50°C to obtain a slurry of 31.08 g corresponding to a percentage yield of 10.6%.

Experimental design

Twenty female rats were randomly assigned to four groups (A, B, C and D) of five animals each. The animals in group A (control) received 1 ml of distilled water orally for 30 days while

rats in groups B, C and D received equal volume of the extract corresponding to 50, 100 and 200 mg/kg body weight respectively. The rats were sacrificed 24hours post-administration for biochemical analysis. The Wistar rats were sacrificed using anaesthesia and jugular puncturing. The blood was collected into a clean sample bottle. They were quickly dissected and the required organ (uterus) was weighed and collected using cold 0.25M sucrose solution, homogenized with mortar and pestle. The homogenized organ was centrifuged and put in a bottle that was cooled in ice, so as to prevent the denaturation of the enzymes present in the uterus, as described by Akanji and Yakubu (2000).

Phytochemical screening.
P. longifolia leaf extract was screened for phytochemicals using the methods of Trease and Evans (1989) and Sofowara (1993). The analyses were done in triplicates each.

Determination of uterine function indices.
Uterine total protein
Total protein was determined using the method described by Bradford *et al*. (1976). Briefly 100 mg Coomasie Brilliant Blue was dissolved in 50ml 95% ethanol(C_2H_5OH). Thereafter, 100ml of 85% phosphoric acid(H_3PO_4) was carefully added under stirring, before water (H_2O) was added to a total volume of 1L. The solution was filtered and kept at 4°C. For measurement, 100 µL extract and 5 ml of Bradford solution were mixed and incubated for 5 min. A standard curve of BSA (0, 0.0625, 0.125, 0.25. 0.5 and 1 g/L) and absorbance was read at 595 nm.

Uterine glucose
Enzymatic indicator was carried out utilizing reaction quantified by the formation of a pink dye. About 1ml of working reagent (phosphate buffer and enzyme), was added to 1ml of distilled water (blank) and 10ul of sample. Incubation lasted for 10mins at 37^0c for 30mins, the absorbance was estimated within 60mins against the reagent blank.
Glucose concentration (mg/dl) $= \frac{\Delta Asample}{\Delta Astandard} \times$ Standard conc.

Alkaline phosphatase activity
The procedure described by Wright *et al*. (1972) was utilized. The amount of phosphate ester hydrolysed within a given period of time is a measure of the phosphate enzyme. Para-nitro phenyl phosphate (PNPP) is hydrolysed to para-nitrophenol and phosphoric acid at pH of 10.1 by the enzyme. The para-nitro phenol confers a yellowish colour on reaction mixture and its intensity is measured at 400nm.
p-nitro phenyl phosphate + $H_2O \xrightarrow{ALP}$ phosphate + p-nitro phenol
Into the test tube, 2.2ml of carbonate buffer (0.1M), 0.1ml of $MgSO_4.7H_2O$ (0.1M) and 0.2ml of appropriately diluted tissue supernatant were mixed and incubated at 37^0C for 10minutes. Afterwards, 0.5ml of p-nitro phenyl phosphate (10mM) was added and the resulting mixture was mixed and incubated at 37^0C for 10minutes. Thereafter, 2.0ml of NaOH (1N) was added to the mixture. The resulting mixture was allowed to stand and the absorbance was read at 400nm against a blank of distilled water (0.2ml). Enzyme activity was calculated using the following expression:

$$\text{Enzyme activity (nmol/min/ml)} = \frac{\Delta\ A/min \times 1000 \times TV \times F}{18.8 \times SV \times L}$$

Where:
Δ A/min = Change in Absorbance of the reacting mixture per minute
TV = Total volume of the reaction mixture.
F = Dilution factor.

SV = Volume of enzyme source.
L = Light path length (cuvette width: 1cm).
18.8 = Extinction co-efficient of 1µm of p-nitro phenol in alkaline solution of 1ml volume and 1cm light path length.
1000 = The factor introduced to enable the enzyme activity to be expressed in nmol/ml.

$$\text{Specific enzyme activity (nmol/min/mg protein)} = \frac{\text{Enzyme activity}}{\text{Protein Concentration}}$$

Uterine cholesterol

The uterine cholesterol was determined by enzymatic kits based on CHOD-PAD reaction as described by Fredickson *et al*. (1967). A known volume (0.02ml) of approximately diluted sample was added to 2.0ml of the working reagent. The blank was constituted by replacing the sample with 0.02ml distilled water and the standard solution. The mixture was incubated at 37^0C for 5minutes after which the absorbance was read on the spectrophotometer, at 546nm. Cholesterol concentration was calculated using this equation:

$$\text{Concentration (nmol/L)} = \frac{\Delta A \text{ Sample} \times \text{Standard conc.}}{\Delta A \text{ Standard}}$$

Where:
ΔA Sample = Change in absorbance of sample
ΔA Standard = Change in absorbance of standard

Data analysis

Data were means ± SEM of five replicates. All results were analysed using one-way ANOVA and Duncan's Multiple Range Test using SPSS version 16. Differences were considered statistically significant at $P<0.05$

Results

Phytochemical constituents

The result of the qualitative analyses carried out on the aqueous extract of *P. longifolia* leaves revealed the presence of saponins, flavonoids, tannins, phenolics, alkaloids, anthraquinones cardiac glycosides, steroids, phlobatannins and cardiac glycosides, while terpenoids were not detected as shown in (Table 1)

Table 1: Phytochemical constituents of aqueous extract of *Polyalthia longifolia* leaves

Phytochemicals	Inference
Saponins	+
Flavonoids	+
Tannins	+
Alkaloids	+
Anthraquinones	+
Steroids	+
Cardiac glycosides	+
Phlobatannins	+
Phenolics	+
Terpenoids	-

Table 2: Effects of the administration of *P. longifolia* leaves on some uterine function indices.

Group/Parameters	A(control) (distilled water)	*Polyalthia longifolia* leaves extract		
		B (50mg/kg)	C (100mg/kg)	D(200mg/kg)
Uterine ALP activity (U/L)	8.60 ± 0.74[a]	9.10 ± 0.78[a]	2.00 ± 1.06[b]	3.08 ± 0.28[b]
Uterine Glucose (mg/dl)	31.71± 4.88[a]	23.39 ±2.02[b]	26.84 ±3.63[b]	24.60 ± 2.71[b]
Uterine Total protein (mg/ml)	5.96 ± 0.81[a]	3.82 ± 0.68[b]	2.10 ± 0.34[c]	1.40 ± 1.66[d]
Uterine Cholesterol (mg/dl)	21.98±1.50[a]	21.98 ±3.50[a]	20.71 ±1.95[a]	15.06 ± 1.56[b]

[1]Values are mean of 5 Determination ± SEM. Row values are mean of 5 Determination ± SEM. Row values with different superscript are significantly ($p<0.05$) different from the control.

Discussion

Phytochemicals are biologically active compounds, found in plants in small amounts, which are not established as nutrients nevertheless contribute significantly to protection against degenerative diseases (Loliger, 1991; Omale and Okafor, 2008). Alkaloids are the most efficient therapeutically significant plant substances commonly found to have antimicrobial properties due to their ability to intercalate DNA of the microorganisms (Kasolo *et al*., 2010). Analgesic, bactericidal and antispasmodic effects have all been attributed to alkaloidal composition in plants (Ganellin and Roberts, 1993; Babajide *et al*., 1999). The presence of alkaloids may be attributed to the acclaimed antibacterial property (Faizi *et al*., 2003ab; Faizi *et al*., 2008) of this plant. Tannins have been suggested to be involved with antibacterial and anti-viral activity while tannins and flavonoids are thought to be responsible for antidiarrheal activity (Adisa *et al*., 2004; Enzo, 2007; Adisa *et al*., 2010). Saponins are known to possess both antimicrobial (Soetan *et al*., 2006) and anti-inflammatory activities (Hassan *et al*., 2012). Studies have also reported the beneficial effects of saponins on blood cholesterol levels and stimulation of the immune system (Cheeke, 2000). Flavonoids have been shown to have antifungal activity in vitro (Galeotti *et al*., 2008). The potent antioxidant activity of flavonoids reveals their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxyl radicals; this may be the most important function of flavonoids (Alan and Miller, 1996). They also induce mechanisms that may kill cancer cells and inhibit tumor invasion (Williams *et al*., 2004). The flavonoids present may be responsible for the medicinal properties accorded the plant (Chen *et al*., 2000; Faizi *et al*., 2003; Saleem *et al*., 2005; Chang *et al*., 2006). Phenols are potent antioxidants which reduces the level of oxidative effect on plant. Plant derived phenolic compounds have been shown to inhibit the initiation and progression of cancers by modulating genes regulating key processes such as oncogenic transformation of normal cells, growth and development of tumors and angiogenesis and metastasis. (Hollman, 2001). The cardiac glycosides have therapeutic ability to increase the force and rate of heart beat without increase in the amount of oxygen required by heart muscles. They can thus increase the efficiency of the heart and at the same time stabilize excess heart beats without strain to the organ (David, 1983). These chemical components found in the aqueous extract of this plant may therefore may be responsible for the claimed therapeutic effect and is also an indication of preliminary validation of the claims. Alkaline phosphatase in tissues and blood are marker enzymes which are used to assess the integrity of the cell membrane, cytosolic activity and

cell death (Akanji *et al*., 1993). Alkaline phosphatase contributes to uterine receptivity, implantation and decidualization (Lei *et al*., 2013). However, low alkaline phosphatase activity, causes diseases like hypophosphatemia (Salina, 2015). Following the administration of aqueous extract of *P. longifolia,* there was a significant decrease in ALP activity at 100 and 200mg/kg body weight extract of the rats when compared with the control. Hence, this might be responsible for blood uterine hypophosphatemia. Studies carried out by Frovola and Moley (2011), revealed that adequate glucose uptake and metabolism, is essential for the proper differentiation of the uterine muscles toward a receptive state, capable of supporting embryo implantation. However, administration at all doses of the extract, caused a significant reduction in uterine glucose concentration when compared with the control which might be implicative of poor embryo implantation, as proper uterine endometrium differentiation might be impaired. Generally, proteins are essential for the transport of nutrients to and from cells, as well as the control of blood clotting around the placenta and uterus. Total protein measurement is used in the diagnosis of variety of diseases involving the liver and the kidney as well as other metabolic diseases (Rao, 1995). Uterine protein was significantly decreased at all dosage administration of the extract, in comparison with the control. Implications of this, might be reduction in nutrient transport and uncontrolled blood clotting around the uterus. In addition, decreased cholesterol, increases uterine activity, as it may be one of the mechanisms operating to maintain uterine quiescence throughout gestation (Smith *et al*., 2005). Administration at 200mg/kg body weight extract, caused a significant decrease in uterine cholesterol compared with the control and the rest of the treatment groups which is an indication that *P. longifolia* has hypolipidemic activity and potential against cardiovascular risk factor. This could mean that uterine activity was probably increased, as a result.

Conclusion

Overall, the phytochemical screening of the aqueous extract of *Polyalthia longifolia* leaves, indicated the presence of five metabolites which were, saponins, flavonoids, tannins, phenolics, and cardiac glycosides. However, in relation to the uterine function indices, aqueous extract of *P. longifolia*, at almost all doses, seemed to have negative effects on the uterine parameters analysed as there was a reduction in ALP activity, uterine glucose, uterine total protein and increased uterine cholesterol (except at 200mg/kg body weight extract). This could imply that the aqueous extract of this plant in relation to the uterus, reduced the functional capacity of the uterine function indices assessed.

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