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Antifertility activity of *Polyalthia suberosa* in male and female wistar albino rats

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A B S T R A C T

To evaluate the potential spermatotoxic effect of ethanolic extract of *Polyalthia suberosa* in male albino rats. Ethnolic extract of *Polyalthia suberosa* were given by gavage to rats in the *in vivo* test at a dose of 200 and 400mg/kg of bodyweight, along with normal controls and their by studying changes in sperm morphology consisting counts, motility and abnormalities of cauda epididymal sperm adapting light microscopy. Finding of this study revealed, the sperm concentration in the epididymis and sperm motility are decreased, whereas sperm abnormalities increased like sloughing of sperm neck, detached head, and coiling of end tail. In extract treated rats, the duration of sperm motility reduced with respect to the increased dose level. A highest 2.09-fold ($p < 0.005$) and 1.28-fold ($p < 0.001$) reduction in the sperm counts and the viability, respectively were observed in *Polyalthia suberosa* 400mg/kg body weight group. Sperm abnormality was increased by *Polyalthia suberosa* in a dose-dependent manner was assessed by acridine Orange fluorescent staining and there was a highest elevation by 2.2-fold ($p < 0.001$) in 400mg/kg group .This result indicates disruption of spermatogenic as well as androgenic compartment. The present study can be concluded the *Polyalthia suberosa* extracts suppress male fertility without altering the general metabolism.

Keywords: *Polyalthia suberosa*, spermatotoxic effect, wistar albino rats, ethanolic extract

A R T I C L E I N F O

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1. Introduction

The future of life on the planet is under the pressure of the population explosion the world's population estimate, for mid-year 2011, is estimated at 6,928,198,253 (Khuranan S *et al*., 1996) and continues to grow by 83 million people per year. During the last half-century, the world's population more than doubled (Herfinadale ET & Gourely DR, 2000). Fertility is an issue of global and national public health concern. Many studies have been done on the male contraception. The traditional use of medicinal plants to treat different sorts of diseases, including fertility related problems is widespread throughout the world as many plant substances are known for their interferences with the male reproductive system (Gupta RS and Rakhi Sharma, 2006). Some of the plants had spermicidal effects; others caused reduction in sperm counts and alter the mobility of the sperms. Some of the plants caused testicular change and altered hormone levels (Bhargava SK, 1984).

Polyalthia suberosa (Annonaceae) is a tropical medicinal plants distributed in the forest of western ghates of India. As per the traditional claims roots are the potential source of drugs for aliments such as asthma, bodyache, bronchitis, fever, cholera dropsy, eye disease, inflammation, malaria, snake bite, rheumatism, tuberculosis wounds and ulcer (Keshavamurthy KR, 1994).

The major groups of chemical constituents present in the *Polyalthia suberos*, the genus has been investigated phytochemically and was reported to contain alkaloids, flavonoids, acetogenin, triterpenoids. Plant mucilage contain polysaccharides. Study yielded an azaanthracene alkaloid, kalasinamide, from the stems of P. suberosa, together with the known N-trans-feruloyltyramine and N-trans-coumaroyltyramine. Study isolated two new 2-substituted furans from the stems of P. suberosa. Leaves contain alpha- and beta-amyrin, lupeol, beta-sitosterol, stigmasterol and campesterol.Stems and leaves contain triterpene, suberosol.Stem bark yields alkaloids, oxostephanine and lanuginosine. Four new styryl-lactones, crassalactones A-D were also isolated.

2. Materials and Methods

Plant material

The aerial parts of the *Polyalthia suberosa* were collected from tirumala hills belong to Tirupati, Andhra Pradesh, India. Taxonomical identifications were made from botanical survey of medicinal plant unit, Sri Venkateswara University Tirupati, Andhra Pradesh. The aerial part of the plant was dried at room temperature, powdered by the mechanical grinder, sieved stored for future used. Exactly 2.5 kg of the fresh air-dried, powered crude drug of *Polyalthia suberosa* was extracted with Ethanol by adopting soxhlet extraction procedure at 60°C for 7 days in a conical flask with occasional shaking and stirring.

Animals

Adult male albino rats were used in the current study. Animals were housed under 12 h light/12 h dark cycle with controlled conditions (21 ± 2^0C, 51 ± 7% humidity) and were fed by standard food (Sai durgs feeds, Bengaluru, India)and allowed water *ad libitum*. Food pellets was with-held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of

Experimental design and treatments

Twenty healthy male albino rats were selected and divided in to four group containing 5 rats each and treated as follows:

Group-I: Received distilled water as normal vehicle (DW)
Group-II: Received as ELEPS (200mg/kg body weight)
Group-III: Received as ELEPS (400mg/kg body weight)
Group-IV: Received as Standard drug lonidamine (50mg/kg body weight) Distill water (DW) ELEPS extracts, 200 & 400mg/kg body weight, was administered Oral route on consecutive 30 days. At the end of the treatment, animals were sacrificed by cervical dislocation and cauda epididymis, vas defenses were immediately dissect out. Sperm from cauda epididymis were released in phosphate buffer saline, followed by Sperm analysis was performed.

Semen Analysis

Dilution of semen: The cauda epididymal duct on one side was exposed and incised. The connective tissue capsule around the epididymis was teased out and the duct was uncoiled. The seen that oozed out into the cavity block is quickly sucked into a capillary tube up to 0.05µl mark and transferred to a 2 ml vial. It is diluted 5ml in phosphate buffer saline. After through mixing by blowing air throw blowpipe the sperm suspension is used for analysis, the *Polyalthia suberosa treated* was observed through sperm motility, sperm morphology and sperm count.

Epididymal Sperm Count and Sperm Motility

The Epididymal sperm suspension is prepared in 1 ml of phosphate buffered saline (PBS) at pH 7.2. The sperm count was determined in a hemocytometer. An aliquot from the suspension (1 ml) was diluted 1:40 with PBS. A sample of the diluted suspension is charged into a counting chamber (Neubauer's chamber). The total sperm count in eight squares (Except the central erythrocyte area) of 1 mm^2 each was determined and multiplied by 5×10^4 to get the total count. Sperm motility was also determined in same eight squares and percentage of motile sperms was recorded.

Acridine Orange and Ethidium Bromide (AO/EtBr) staining of Sperm:

In order to find the viability of spermatozoa, fresh sperm were stained with acridine orange (AO) and ethidium bromide (EB). A fine suspension was made and stained with 25µl of AO-EtBr. About one drop of stained suspension was placed on the clean slide and allowed to dry. The preparations were observed in the same microscope, now with epifluorescent attachment. In all cases the images were captured in a Sony DXC-151AP CCD camera (Tokyo, Japan). In all cases of counts of spermatozoa with morphological abnormalities, 200 randomly selected spermatozoa from each slide were observed and assigned to the categories viz., normal, head alone and flagellar defect of interest in this study.

Statistical analysis: The data expressed as mean ±SEM and analyzed by One-way analysis of variance. $P<0.05$ was considered as the criterion for statistical significance.

3. Results and discussions

Results

Effect of *Polyalthia suberosa* on sperm count

In the vehicle control rats had normal sperm counts, motility, and morphology (Table 1). In *Polyalthia suberosa* treated rats, the cauda epididymal sperm parameters showed evidence of dose dependent infertility. The sperm counts were significantly decreased in group II group III and group IV animals compared to that of vehicle control animals. (Fig.1). A highest 2.09 fold ($p<0.005$) and 1.28-fold ($p<0.001$) reduction in the sperm counts and viability respectively were observed in *Polyalthia suberosa* 50 mg/kg b.wt group. So the group IV animals (Table .1) the sperm counts were highly reduced when compared to that normal control.

Effect of *Polyalthia suberosa* on sperm motility

In vehicle control treated rats, cauda epididymal sperm revealed rapid progressive motility and it was lasted for about 1 hr 50 min. But, in rats treated *Polyalthia suberosa* 200 mg/kg (group-II) sperm was progressive for 30 min. On the other hand, in rats treated with *Polyalthia suberosa* 400 mg/kg (group-III) sperm were sluggish for 12 min. and *Polyalthia suberosa* 400mg/kg (group-IV) sperm were not at all motile. The sperm motility was highly inhibited in group II group III group IV animals.

Effect of *Polyalthia suberosa* on sperm morphology

More than 50% of the sperm had abnormal morphologies of various kinds, which included abnormalities in the neck and tail of the sperm (bend neck), coiling of sperm, DNA damage sperm, sloughing of the sperm neck and fragmentation of head were observed by *Polyalthia suberosa* extracts in a dose-dependent manner. Which are also assessed by Acridine Orange fluorescent staining there was a highest elevation by 2.2-fold ($p < 0.001$) in 500mg/kg group (Table. 1).

The plant extraction intoxication exerted a significant decrease in epididymal sperm concentration and sperm progress motility. The live/ dead sperm count was increased in group II group III group IV animals. The reduction of sperm count and sperm motility were significantly ($P<0.05$) higher in group IV treated animals when compared to that of vehicle control.

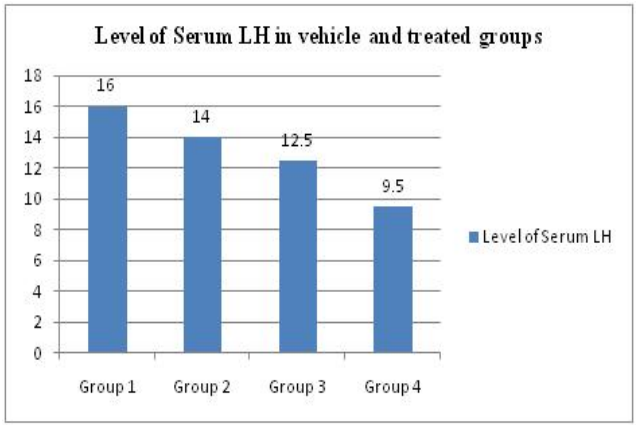

Figure 1: Estimation of LH in rat serum collected from Control and treated animals

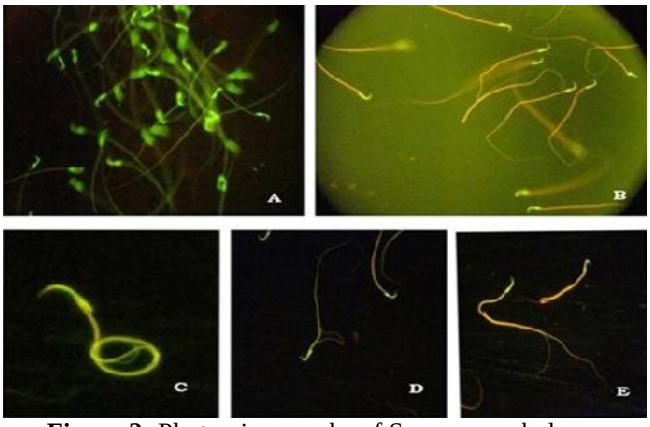

Figure 2: Photomicrographs of Sperm morphology

Photomicrograph of sperm morphology represents:

A-Vehicle Control animal has normal morphology showing intact head and tail

B-ELEPS 200mg/kg.bw shows abnormalities in the neck and tail of the sperm (bend neck)

C-ELEPS 400mg/kg.bw shows Coiling of sperm and sloughing of sperm neck present

D and E- Standard drug lonidamine 50mg/kg.bw shows fragmentation of head (increased red or orange fluorescence)

Discussion

Medicinal plants have increasingly become an integral part of the human society in combating various diseases, ranging from skin infection to gastrointestinal problems, since the dawn of civilization (Gu ZP *et al*., 2000). The present study indicates that *Polyalthia suberosa* treatment result in impairment of male fertility in the rat by affecting both Spermatogenesis and cauda epididymal spermatozoa. Among, plant based contraceptives; inhibition of male fertility after administration of natural substance has been related decreased spermatozoa density (Sharma N & Jacob D, 2001). Also for male contraceptive, it is not necessary to stop spermatogenesis but rather to eliminate the fertilizing ability of spermatozoa by causing changes in morphology or in the function of sperm (Nikkanen V *et al*., 2000).

It is known as structure and function if epididymis is dependent upon the androgens. Because of androgen deprivation causes the suppression of spermatogenesis leading to a low sperm concentration and alters the epididymal milieu (Srivastav A, 2000). In present investigation a dose dependent suppression of epididymal sperm motility and sperm count suggested may be under supply of testosterone to the epididymis. Sperm motility and high velocity are important for sperm binding to and penetration in to the *Zona pellucida* for successful fertilization (Bauer JD *et al*., 1974).

The breaking away of head from flagella and flexion of the head of the sperm appears to occur due to impact of active chemicals of *Polyalthia suberosa* at the neck or connecting piece of flagellum. The main components of connecting piece are the basal plate, capitulum and segmented column. Trypsin treatment appears of cleave the head from the tail

between capitulum and basal plate (Robaine B and Hemo L, 1988). Thus, it could be perceived that the Ethnolicextracts disrupt the protein also as much as disrupting tubulin, causing the breaking away of the head from flagellum. A less impact at this point could cause the head to flex or flexion itself may be a step towards the breaking away (Young RJ and cooper GE, 1983). A decrease in human sperm motility might be due to mitochondrial disruption and/ or an increase in lipid peroxidation. The flagella movement of the sperm cells decides the motility of cells and energy to flagella which is provided by the mitochondria (Nakai M *et al*., 1992).The reduction of sperm motility and density in cauda epididymis is of important correlates with fertilization (Bedford JM, 1983). Alteration in the sperm viability, motility and morphology suggest a disturbed testicular and epididymal microenvironment. Because of adequate number of sperm s possessing normal function is necessary for successful fertilization, any deviation that alters sperm function leads to infertility (Carft I *et al*., 1993). Inadequate concentration, sluggish or non-motile spermatozoa could not penetrate the cervical mucus and thus failed to fertilize the ova (Manivannan B *et al*., 2009; Pankajakshy A & Madambath I, 2009).

Lack of motility, decrease sperm count, increase incident of sperm abnormalities strongly point to a spermatotoxic effect of *Polyalthia suberosa* via epididymis, particularly tail coilly nature of the sperm suggested some biochemical change in the sperm surface. The Ethnolicextract of aerial part of *Polyalthia suberosa* at a treated dose of 300 and 500 mg/kg arrested normal spermatogenic cycle and showed increase sperm abnormalities.

Table 1: Effect of ELEPS on Sperm concentration, Motility and Morphology

Groups	Treatment	Total sperm count		Sperm motility	Abnormal Sperm morphology
		Head	Tail		
Group 1	Saline	98.35±3.49	92.27±3.13	99.74±6.24	3.84±0.24
Group 2	ELEPS (200mg/kg)	73.52±11.32	72.47±9.36**	73.53±9.68**	31.64±7.54***
Group 3	ELEPS (400mg/kg)	52.24±13.59	54.13±12.49***	57.52±13.48***	38.04±6.43***
Group 4	STD (50mg/kg)	34.16±6.24	32.74±7.13***	35.21±7.34***	54.32±4.04***

Values represent the Mean±SD of the observation made on five rats in each group. Statistical analysis one way analysis of variance (ANOVA) with hoc testing least significant difference.

4. Conclusion

The present study indicates that *Polyalthia suberosa* responsible for the aspects of male antifertility effect. Further long term studies are in progress for the evaluation of complete and reversible fertility with this extract and also other effects of this important plant.

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