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Research Article

FERTILITY ENHANCEMENT OF *POLYCARPAEA CORYMBOSA* (L.) LAM (CARYOPHYLLACEAE) WHOLE PLANT ON MALE ALBINO RATS

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ABSTRACT

Objective: To evaluate fertility enhancement activity of ethanol extract of whole plant of *Polycarpaea corymbosa* in male albino rats.
Methods: Two groups (Group II & III) of rats were administered orally ethanol extract of whole plant of *Polycarpaea corymbosa* at doses of 250 and 500mg/Kg body weight. The control group (Group I) received normal saline. After 14 days, sperm count, sperm motility, biochemical parameters, hormone levels, the number of implantation sites, the number of fetuses and the number of resorption sites were recorded.
Results: The relative weight of the testes and epididymis were increased. The epididymal sperm count, motility and sperm abnormality were increased significantly in treated rats. There was an increase in serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of treated rats. The activities of serum antioxidants (CAT, SOD, GPX, GST and GRD) in whole plant extract treated rats were increased. The results of the hormonal assay showed that increased serum levels of LH and testosterone but decreased in the serum levels of FSH and estrogen compared to control. The results of fertility test indicated that the treated adult male rats increased the number of female's impregnation. In addition, the number of implantations and the number of viable fetuses were also increased.
Conclusion: The results of the present study concluded that, ethanol extract of whole plant of *Polycarpaea corymbosa* enhanced sperm concentration, motility and testosterone which might produce positive result in a male fertility.

Keywords: *Polycarpaea corymbosa*, Fertility, Testosterone, Antioxidant

INTRODUCTION

The use of herbs is very common in developing countries, particularly in rural settings. However, during the last decade an increase in the use of plants has been observed in metropolitan areas of developed countries. Plants are extensively used to relieve sexual dysfunction. Folk remedies have long been advocated, with some being advertised widely since the 1930s [1]. There are many herbal drugs that have been used by men with erectile dysfunction with varying degrees of success. Most potent herbal aphrodisiacs are available and have little or very little side effects [2-4].

Polycarpaea corymbosa (L.) Lam. Belongs to 'Caryophyllaceae" is commonly known as "Pallipoondu" in Palliyar tribals of Sirumalai hills, Western Ghats, Tamil Nadu. Paste prepared from the whole plant is taken once in a day for a period of 2-3weeks to treat jaundice by the Palliyars [5]. Biological activities such as anticancer, antiulcer, antidiarrhoeal, anti-inflammatory, antioxidant and hepatoprotectivity were reported [6-11]. In the light of the above findings, this work was conducted to monitor its effect on reproductive system and fertility in adult male rat.

MATERIALS AND METHODS

Plant materials

Whole plant of *Polycarpaea corymbosa* (L.) Lam was collected from Sirumalai hills, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract

The whole plant of *Polycarpaea corymbosa* were dried separately under shade and then powdered with a mechanical grinder to obtain a coarse powder, which were then subjected to extraction in a Soxhlet apparatus using ethanol. The ethanol extract were concentrated in a rotatory evaporator. The concentrated ethanol extracts of whole plant of *Polycarpaea corymbosa* were used for preliminary phytochemical screening [12] and anti-fertility activity.

Animals

Normal healthy male Wistar albino rats (180-240g) were used for the present investigation. Animals were housed under standard environmental conditions at temperature (25±2^{0}C) and light and Dark (12:12h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats of either sex selected by random sampling were used for acute toxicity study [13]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric incubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

Experimental Design

The male rats were divided into 3groups consisting of 5 animals.

Group I : Rats received normal saline daily for 14 days, orally. (Normal control).

Group II: Rats received ethanol extract of whole plant of *Polycarpaea corymbosa* at the dose of 250mg/kg body weight daily for 14 days.

Group III: Rats received ethanol extract of whole plant of *Polycarpaea corymbosa*, at the dose of 500mg/kg body weight daily for 14 days.

After 24 hours of last treatment, the final weight was recorded and the animals were sacrificed by decapitation. Blood was collected. Sera were separated by centrifugation at 3000g for 10 minutes and stored at 20^{0}C until used for various biochemical assays. Then

testes, epididymis, vas deferens, seminal vesicle and ventral prostrate were dissected out, trimmed off extraneous and weighed accurately on torsion balance. The organs weights were expressed in terms of mg/100g body weight.

Sperm count

Epididymal fluid (for sperm count) was collected from caput and cauda segments separately and diluted with Sorenson's buffer (pH7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer as described by Zaneveld and Pelakoski [14].

Sperm motility and abnormality

After anaesthetizing the rats, the caudal epididymis was then dissected. An incision (about 1mm) was made in the caudal epididymis and drops of sperm fluid were squeezed onto the microscope slide and 2 drops of normal saline were added to mobilize the sperm cells. Epididymal sperm motility was then assessed by calculating motile spermatozoa per unit area.

Morphology (abnormality) was evaluated on sperm from the caudal epididymis. The total morphological abnormalities were observed by the method of Linde *et al* [15].

Serum biochemical analysis

Serum proteins [16] and serum albumins were determined by quantitative colorimetic method by using bromocresol green.

The total protein minus albumin gives the globulin, urea [17], creatinine [18], serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) was measured spectrophotometrically by using standard method [19]. Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong [20].

Serum antioxidants

Serum antioxidant Catalase (CAT) [21], Superoxidedismutase (SOD) [22], Glutathione peroxidase (GPx) [23], Glutathione S-transferase (GST) [24] and Glutathione reductase (GRD) [25] were analyzed .

Hormonal Assay

Blood removed from the rats by intracardiac method. Blood was centrifuged at 3000 rpm to separate the serum for the measurement of testosterone, Luteinizing hormone (LH), estrogen and follicle stimulating hormone (FSH). The quantitative determination of hormones was done by using Enzyme Immunoassay Method (EIA). The EIA kit was obtained from Immunometrics (London, UK).

Fertility test

Fertility was estimated in adult male rats treated with ethanol extracts of whole plant of *Polycarpaea corymbosa* and in the control male counterparts. Each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days during which two estron cycles had elapsed. One week after the removal of the exposed males, pregnant females were killed by cervical dislocation under light ether anesthesia and the number of implantation sites, the number of fetuses and the number of resorption sites were recorded [26].

Statistical Analysis

Data were expressed as Mean ± SEM. Student's t test was used for statistical comparison.

RESULTS

Preliminary phytochemical screening and acute toxicity studies

Phytochemical screening of ethanol extract of whole plant of *P. corymbosa* revealed the presence of alkaloids, catechin, coumarin, tannin, phenols, saponins, steroid, flavonoid, glycoside and xanthoprotein. The acute toxicity study, ethanol extract of *P. corymbosa* whole plant did not show any toxicity effect upto the dose of 2000mg/kg body weight, according 250 and 500 mg/kg body weight were taken as low and high dose of whole plant of *P. corymbosa* for the experiment.

Body weight and reproductive organ weight

Table-1 shows slight increase in the body weight after administration of the whole plant extract of the *P. corymbosa* while the weight of the testes, epididymis, seminal vesicle, ventral prostrate and vas deferens were significantly increased (P<0.01) in treated male rats compared to control group (Group-I).

Table 1: Effect of whole plant extract of *Polycarpaea corymbosa* on the body and reproductive organ weight of adult male albino rats.

Treatment Groups	Body wt(gm)		Testis (gm)	Epididymis (mg)		VD (mg)	SV (mg)	Prostrate (mg)
	Before	After		Caput	Cauda			
Group-I	259.56±9.43	278.50±8.92*	1.914±0.38	126.33±3.04	284.56±2.44	116.39±2.84	294.11±6.85	163.22±2.94
Group-II	224.54±5.43	238.62±4.93	1.989±0.73ns	213.84±2.98**	324.83±2.16**	124.16±2.84	293.91±3.88	183.54±1.98ns
Group-III	284.41±6.93	299.22±7.84**	2.093±0.67**	224.66±3.05**	344.87±4.26**	136.63±1.98*	311.54±4.93*	198.36±3.94**

Each Value is SEM of 5 animals * P < 0.05; ** P < 0.01. Control vs Treated, ns- not significant

Sperm density and motility

Table-2 shows that the motility of sperm in cauda epididimis was significantly increased (P<0.01) *P. corymbosa* whole plant extract treated rats in comparison with control. Sperm density in treated animals was increased significantly. The sperm abnormality was decreased significantly in the treated animals.

Table2: Effect of whole plant extract of *Polycarpaea corymbosa* on the sperm concentration and motility in the epididymis of adult male albino rats.

Treatment Groups	Sperm Concentration (Counts x 10^6 mil)		Sperm Motility (FMI) @ (cauda)	Sperm Abnormality #	
	caput	cauda		Head (%)	Tail (%)
Group-I	356.24±10.84	419.54±15.36	153.26±10.36	5.94±0.18	8.93±0.34
Group-II	363.54±9.31ns	424.54±10.24ns	163.91±5.41	4.27±0.18	6.54±0.84
Group-III	379.08±11.27*	461.33±12.98*	182.36±2.63*	3.01±0.021*	4.16±0.63*

Each Value is SEM of 5 animals * P < 0.05 Control vs Treated, ns- not significant

@ : Motility is movement recorded after 5 min in the suspension of caudal epididymal spermatozoa in phosphate buffered solution. # : Expressed in percentage

Serum biochemical profile

Serum protein, albumin, globulin, urea, creatinine and the activity of liver marker enzymes (SGOT, SGPT and ALP) levels of control and treated rats were depicted in table-3. All the parameters were significantly increased.

Table 3: Effect of whole plant extract of *Polycarpaea corymbosa* on the activity of serum catalase, glutathione peroxidase, glutathione-s-transferase, superoxidedismutase and glutathione reductase in rats.

Treatment	Catalase (µmoles of H2O2/decomposed/ min/mg protein)	Glutathione peroxidase (µmoles of NADPHoxidized/min/ mg protein)	Glutathione-Stransferase (µmoles ofconjugate formed/	Superoxide dismutase (Units)	Glutathione reductase (µmoles of NADPH oxidized/min/
Group I	7.93±0.24	0.274±0.03	10.14±0.93	22.66±1.85	28.04±1.31
Group II	7.84±0.34	0.294±0.11ns	12.41±0.76ns	27.21±1.06ns	38.22±0.93*
Group III	9.95±0.16*	0.365±0.24*	14.81±0.24*	29.68±1.13**	42.18±0.72*

Values are given as means ± S.D from six rats in each group * *P* < 0.05, ** *P*<0.01 Control vs Treated. ns- not significant

Serum antioxidants

The activities of CAT, SOD, GPx, GST and GRD in the serum of control and whole plant extract treated rats were presented in table-4. In the present study, plant extract treated rats had shown increased activities of all the studied antioxidants when compared to control rat.

Table 4: Effect of whole plant extract of *Polycarpaea corymbosa* on few serum biochemical profile of adult male albino rats.

Parameter	Group I	Group II	Group III
Protein (gm/dl)	7.11±0.24	8.06±0.14	8.74±0.93*
Albumin (gm/dl)	4.65±0.65	4.31±0.74	4.33±0.68
Globulin(gm/dl)	2.46±0.12	3.75±0.66	3.81±0.27
A/G Ratio:	1.89:1	1.14:1	1.14:1
Urea(mg/dl)	15.33±0.52	21.88±0.36	23.63±1.08
Creatinine(mg/dl)	0.63±0.03	0.94±0.05	0.89±0.03
SGOT (U/L)	11.84±1.08	29.14±1.93	34.56±2.08
SGPT(U/L)	15.36±2.94	30.54±2.29	32.54±2.08
ALP(U/L)	167.55±4.86	194.33±5.54*	202.14±2.34*

Values are given as means ± S.D from six rats in each group * *P* < 0.05 Control vs Treated.

Reproductive hormone level

Serum testosterone level

The ethanol extract of whole plant of *P. corymbosa* (250 and 500 mg/kg body weight) repeated treatment for 14 days caused significant increase in serum level of testosterone in male rats. The level of testosterone increase was dosing related (Table-5).

Serum luteinizing hormone (LH) level

Repeated treatment of male rats with the ethanol extract of whole plant of *P. corymbosa* for 14 days caused a dose related increase in the serum level of LH.

Serum estrogen level

The ethanol extract of whole plant of *P. corymbosa* caused decrease at dose related serum level of estrogen when compared with control.

Serum follicle stimulating hormone (FSH) level

Pre-treatment with the ethanol extract of whole plant of *P. corymbosa* caused decrease in the serum level of FSH male rats compared to control.

Table 5: effect of whole plant extract of *Polycarpaea corymbosa* on sex hormones levels and pituitary gonadotrophins in male albino rats

Treatment Groups	Parameters			
	Testosterone (mg/ml)	LH/ICSH (µIu/ml)	Estrogen(pg/ml)	FSH (µIu/ml)
Group I	3.03±0.85	1.98±0.05	18.31±0.24	0.98±0.05
Group II	3.12±0.85	2.07±0.51	17.56±0.23	1.91±0.05
Group III	3.95±0.31*	2.48±0.08*	15.11±0.56*	1.86±0.03*

Values are given as means ± S.D from six rats in each group * *P* < 0.05 Control vs Treated.

Fertility test

The results presented in table-6 show that intra-gastric administration of the ethanol extract of whole plant of *P. corymbosa* at doses 250 and 500 mg/kg body weight for 14 days to male rats caused a significant increase in the number of females impregnated by treated male rats. The number of implantations and the number of viable fetuses calculated after cesarean suctions were significantly increased in female rats impregnated by treated males when compared with female rats impregnated with untreated male rats. On the other hand, the number of resorptoin sites were found to be increased to significant values in female impregnates by treated male rats when compared to controls.

Table 6: effect of whole plant extract of *Polycarpaea corymbosa* on the fertility of male albino rats

Groups	No. of male	No. of females	No. of pregnant females	No. of implantation	No. of viable fetuses	Total No. of resorption sites
Group-I	2	6	5/6	9.31±0.84	4.18±1.26	4
Group-II	2	6	4//6	5.84±0.16	5.16±0.94	4
Group-III	2	6	6/6	8.55±0.34	6.27±0.23*	5

Each Value is SEM of 5 animals * *P* < 0.05, Control vs Treated

DISCUSSION

In the present study, the weight of reproductive organs markedly increased. The weight and secretary functions of testes, epididymis, seminal vesicles, ventral prostrate and vas deference are closely regulated by androgens. The drug may act on pituitary gland and increased main hormone of spermatogenesis. It is well established fact that weights and secretary functions of the epididymis, seminal vesicle and ventral prostrate are closely regulated by the androgens, changes taking place in these organs after castration can be counteracted by administration of testicular hormones thus serving as "indicator test" for the male hormones [27, 28]. The results presented in this work also show that the seminal vesicles weights were increased in adult male rats ingested *P. corymbosa*. This increase in the accessory glands weights might suggest an increase in the pattern of testosterone secretion. Significant increase in the sperm motility of cauda epididymis was observed in treated group. This may be due to activity effects of *P. corymbosa* on the enzymes of oxidative phosphorylation.

Sexual cells can occur during the reproductive phase, mitotic division of the spermatogenesis or during the maturation of the spermatozoa, thereby increasing the number and quality of the sperm cells produced in the testes. Among the ethanol extract of *P. corymbosa* whole plant (Group-II and III) (250 and 500 mg/kg body weight) produced a significant increase in total sperm count and viable sperms. This may be as a result of the ability of the extract at the given doses, to either interfere with spermatogenetic process in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadodropins which may result in alteration of spermatogenesis. The presence of mature sperm concentration was increased in the experimental rats treated with 500 mg/kg body weight *P. corymbosa* whole plant extract. This suggests that the 500 mg/kg dose could influence the maturation of the spermatozoa in the male rats, which might also be a contributory factor to the increase in the mean total sperm count.

In the present investigation the observed increase in the cauda epididymal sperm motility might be due to an alteration in the microenvironment in the cauda epididymis, which also had a synergistic action on the spermatozoa of the treated rats as a result of the androgen-stimulatory effect of the extract of *P. corymbosa* whole plant. The increase in the cauda epididymis sperm counts in the treated animals substantial the spermatogenic nature of the extract. The extract had a direct effect on the testes resulting in an increase in the number of spermatozoa and the increased level of testosterone production. Generally, elevated testosterone level also enhances the sexual behavior in humans. Therefore, an increase in testicular and serum free testosterone concentration will confirm fertility enhancement potential inherent in the plant extract. Also, the extract had no spermatotoxic effect as previously indicated by Shah *et al* [29].

Luteinizing hormones (LH) and Follicle Stimulating Hormone (FSH) produced by anterior pituitary lobe are necessary for maintaining testosterone levels such that as LH and FSH increases so do the testosterone. Therefore, a medicinal plant acclaimed to have aphrodisiac potential apart from being able to increase the concentration of bioavailable/free testosterone should cause increase in the concentrations of serum LH and FSH. An increase in the concentrations of LH and FSH should normally increase the testosterone concentration. The increased level of superoxide dismutase, catalase, glutathione peroxidase, glutathione S-tranferase and glutathione reductase were reported in the present study. Similarly, total protein, SGOT, SGPT and ALP levels were increased in the serum of extract treated rats.

The extract did not show an antigonadotrophic nature, demonstrated by the increased levels of FSH and LH in the treated rats. The increased level of FSH reveals a possible role of *P. corymbosa* whole plant extract in influencing the release of gonadotrophic hormones from the pituitary. The rise of FSH by itself is of critical importance in the initiation and expansion of spermatogenesis in mammals, as is generally agreed [30]. The results presented in this paper also show that the ingestion *Polycarpaea corymbosa* by adult male rats increased the number of impregnated females. The number of implantations and the number of viable fetuses were increased. This effect may be due to increase in sperm motility and sperm density.

In conclusion, these results confirmed that the long term *P. corymbosa* ingestion produces increased effects on fertility on reproductive system in adult male rat. However, the exact mode of action requires further studies.

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