

Biochemical Assessment of Fertility-Enhancing Effect of Aqueous Extract of *Symphonia globulifera* (Linn. F.) In Adult Male Wistar Rats

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Abstract

The present study investigated the effect of oral administration of aqueous extract of *Symphonia globulifera* root on fertility in addition to its effect on some biochemical indices in adult male Wistar rats. After the aqueous extraction, phytochemical screening was carried out. Twenty-four (24) mature and healthy adult male wistar rats of weights between 150-220 g were used. The rats were randomly divided into four (4) groups each containing six (6) rats. Group 1 rats were gavaged with distilled water which served as control group. Group 2, 3 and 4 rats were orally treated with 100, 200 and 400 mg/kg body weight of the aqueous extract of *S. globulifera* respectively for 21 days. After the treatment, the rats were sacrificed and their organs (heart, liver, kidney and testes) were excised. Hormonal analysis which include seminal fluid analysis (SFA), follicle stimulating hormone (FSH), leutenizing hormone (LH), testosterone and prolactin were carried out following standard procedures. The data were analysed using students t-test and ANOVA with the aid of SPSS with $P < 0.05$ level of statistical significance. The phytochemical screening of the extract revealed presence of major phytochemicals. There was dose-dependent decrease in the body weight of the rats after the treatment. In hormonal analysis, SFA increased significantly when compared to that of the control rats. The analysis also revealed that the blood level of follicle stimulating hormone (FSH) was significantly increased whereas; there was decrease in testosterone level when compared with the control animals. In conclusion, aqueous extract of *S. globulifera* was found to be safe and showed significant fertility enhancing effects.

Keywords: biochemical assessment; fertility; aqueous extract; *symphonia globulifera*; adult male rats

Introduction

Infertility is the inability of a couple of opposite sex to achieve a clinical pregnancy after 52 weeks or more of regular unprotected sexual intercourse. In other words, it is a complete failure of a sexually competent and active, non-contracepting couple to achieve pregnancy in one or more years despite regular sexual exposures [1]. It can be caused by low sperm production (oligozoospermia), poor sperm motility (asthenozoospermia), or abnormal sperm morphology (teratozoospermia) poor sperm quality, sperm abnormalities [2]. However, generally, a combination of these, oligoasthenoteratozoospermia (OAT), is considered to be the most common cause of male subfertility [3]. Infertility is a common problem, studies suggest that male problems represent the commonest single defined cause of infertility [4].

The most common manifestation of male infertility is as oligospermia but can also manifest in the form of premature ejaculation, hypoactive sexuality and erectile dysfunction. The management options available for the treatment of infertility in males include the use of drugs and a variety of surgical procedures [5]. The total sperm count, motile sperm count and with normal morphologic features has been reported as the indices of

fertility in males [6]. It was reported that, testosterone and other forms of hormone replacement therapy are often clinically employed in treatment due to their ability to stimulate/enhance sexual appetite in hypogonadal male patients [7]. However, despite the potency of this therapy in the management of infertility [8], some patients still prefer to use plants remedy because of the attendant undesirable side-effects associated with these hormonal therapies. The use of plant extracts as fertility enhancer in animals is now in the increase because of the shifting attention from synthetic drugs to natural plant products [9]. Plants that were once considered of no value are now being investigated, evaluated and developed into drugs, with little or no side effects [10]. The use of medicinal plants as remedies is common and widespread in Nigeria. Currently, the society at large appreciates natural cure, which medicinal plants provide compared to synthetic cure. The plants parts used in remedies include the bark, leaves, roots, flowers, fruits and seeds [11].

Medicinal plants that have been reported to be used in fertility enhancement include; ethanolic extract of *Bulpleurum marginatus*, *Praneem vilii*, ethanolic extract of *Trichopus zeylanicum* and many others. Medicinal plants for years, as sources of improving semen quality

in animals and human being, have been explored for potential fertility enhancing properties [12, 13]. Studies have indicated that herbs and herbal substances containing wide spectrum of bioactive constituents protect the semen characteristics from free radicals that arise out of various stressors [14]. Preparations containing medicinal plants such as *Zingiber officinale*, *Mucuna pruriens*, *Piper longum*, *Tribulus terrestris* are claimed to improve the semen quality in rats [15, 16].

Symphonia globulifera has been widely used in traditional and folklore medicine and has therefore been subjected to several phytochemical studies in the American and African continents [17]. *S. globulifera* known locally as “agba” in Igbo “arhohen” in Urohbo “afato” and “ogolo” in Yoruba is a very popular medicinal plant among herbalists in Southwestern Nigeria because of its acclaimed effect in fertility enhancement properties in men. It has been reported that various part of plant is used in many part of the world to treat itching, stomach ache, skin infection, as laxative, general tonic, chest cough, gonorrhoea, as anti-diuretic, it also stops nose bleeding [18]. *S. globulifera* is a plant native to the Latin America and tropical Africa. It is a timber tree, also used as medicinal and ornamental, due to the beauty of its flowers, known as chewstick. In traditional medicine, it is used against parasitic disorders in Africa [19], in South America and to relief pain. The plant is traditionally used as laxative for pregnant women, in Cameroon [20]. The leaves are toasted and used as cataplasm in wounds derived from snake bite, while stem bark is used in infected wounds, the sap is used in vision-related diseases and decoction made with stem bark is used against cutaneous leishmaniasis [21]. The so acclaimed fertility enhancement property of the plant by traditional herbalists has not been scientifically investigated. The effect of *S. globulifera* is inculcated into this research to investigate the effect of oral administration of its aqueous extract on some serum marker enzymes, Lipid profile, hematological parameters, male hormones, seminal indices, histology, protein metabolism and electrolytes (as indices of liver and kidney function) using animal model. The focus is therefore, to examine the fertility enhancing properties of the root aqueous extract of *S. globulifera* in male wistar rats through analysis of sperm number, sperm motility and morphology and other biochemical parameters.

Materials and Methods

Plant Material

The root of the *S. globulifera* was bought from an herbalist in a local market, Lagos, Southwestern Nigeria. It was identified at the Herbarium, Department of Botany, University of Lagos and Voucher specimen was deposited for further authentication.

Chemicals

The chemicals include kits for hormonal assay: Testosterone, Follicle Stimulating Hormone, prolactin, luteinizing hormone and seminal fluid analysis were purchased from Biotech Laboratory Ltd, United Kingdom.

Also, kits for total and direct bilirubin, glucose, urea, uric acid and creatinine used for the biochemical studies were purchased from BIOLABO REAGENTS, Maizy, France (Version : AT 80107 05/07/2004).

Preparation of Plant Root Extract

Roots of *S. globulifera* were cut, washed and air dried. The dried plants were pulverized into a dry powder. Extraction was carried by soaking 700 g of the powder in 12 L of distilled water. The decoction was filtered with cheese cloth and the filtrate was concentrated in an electric oven at 50 °C until a semisolid residue was obtained. The percentage yield of the extract was calculated using the formular:

$$\text{Yield} = \frac{\text{Weight of Semisolid Residue}}{\text{Weight of Powdered Root}} \times 100$$

Weight portions of semisolid residue were used in the calculations of doses of 100, 200 and 400mg/kg bodyweight.

Phytochemical Analysis

The phytochemical screening of the plant aqueous extract was carried out according to the method of Edeoga et al [22].

Experimental Animals and Study Design

Twenty-four (24) mature and healthy adult male wistar rats of between weights 150 to 220g were used for this study. It was conducted in accordance with the internationally accepted principle for laboratory use and care (US Guidelines [23]. The animals were purchased from a private animal House at University of Ibadan; they were acclimatized in cages and maintained in animal facility of University of Lagos before commencement of the treatment for 21 days. They were given free access to rat feed (commercial rat pellets from Niemeth livestock Feeds, Ltd. Ikeja) and water ad-libitum under normal laboratory environmental conditions. The rats were randomly divided into four (4) groups of six (6) rats each. The rats were gavaged with different doses of the extract for a period of 21 days as follows;

Group 1 were the control group gavaged with distilled water

Group 2 were given 100 mg/kg body weight

Group 3 were treated with 200 mg/kg body weight

Group 4 were given 400 mg/kg body weight

Body Weight Measurement

Rat body weights were measured using digital Mettler weighing balance and values obtained were recorded prior to commencement of the experiment. The body weights of the rats were taken weekly during the treatment period, and on the last day of the experiment.

Experimental Analysis and Methods

After 21 days of treatment, all the animals were anaesthetized with phenolbarbitone sodium and sacrificed. The blood samples were drawn from the heart of each sacrificed animal. The samples for Hematology tests were collected in EDTA plastic bottles and that of Blood Glucose in Fluorite oxalate bottles to prevent clotting. The samples for biochemical tests were collected in plane bottles and allowed to stand for 2 h to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen. The blood film was systematically examined and the different white blood cells seen in each field were counted using a differential cell counter.

Hormonal Analysis

Hormonal analysis was carried out for follicle stimulating hormone (FSH), testosterone, luteinising hormone (LH) and prolactin (PRL) using standard procedures and corresponding commercially available kits. All samples were run in the same assay period. Determination of total proteins was by Biuret method [24]. Determination of serum albumin, uric acid, urea and creatinine were also conducted using the method of Labbe et al [25].

Semen Analysis for Motility, Count, and Morphology

Semen analysis to determine progressive motility, count, and morphology was also carried out following the method of Amman [26]. Number of sperms per cauda epididymis was calculated as follows: Mean count x 50} ÷ 0.01 x 0.01 }.

Histopathological Analysis of the Testes

Histopathological analysis of the testes of the control untreated (group 1) and the treated group (Group 4 with 400 mg/kg body weight) was carried out following established protocol.

Statistical Analysis

All results were analyzed using students t-test and ANOVA with the aid of SPSS (version 15) software package. The level of statistical significance was taken as $P < 0.05$.

Results

Aqueous Extraction

After the concentration in an oven, the percentage yield obtained for the semisolid residue of *S. globulifera* was 10 ± 0.1 %.

Phytochemical Analysis

Phytochemical analysis of the extract showed the presence of terpenoids, flavonoids, tannin, cardiac glycosides, saponin, and steroids. However, phlobatanins was not detected (Table 1).

Effect of Oral Treatment of Aqueous Extract of *S. globulifera* on Body Weights of Rats

Table 2 shows the effect of the extract on the body weight of the treated rats. Repeated oral treatments with 100, 200 and 400 mg/kg/day for group 2, group 3 and group 4 respectively of *S. globulifera* induced significant ($p < 0.05$) dose related decrease in the weight gain pattern of treated rats effective from the day to the day of oral treatment when compared to the progressive weight gain pattern of control rats. There was progressive decrease in the weight but the most significant reduction was observed for Group 4 (400 mg/kg/day body weight of *S. globulifera*).

Effect of Oral Treatment with Aqueous Extract of *S. globulifera* on Organs Weight of the Rats

Oral treatments with 100, 200 and 400 mg/kg/day for group 2, group 3 and group 4 respectively of *S. globulifera* for 21 days caused no significant alteration in the organs weight pattern of treated rats when compared to the organ weight pattern of control rats gavaged with distilled water (Table 3).

Effect of Aqueous Extract of *S. globulifera* on Renal Function Indices in the Male Rats

Table 4 shows the renal function indices of adult male wistar rats treated with aqueous extract of *S. globulifera*. The level of urea and uric acid were observed to increase significantly on group 2 rats treated with 200 mg/kg body weight of the aqueous extract of the plant as compared to the control group. A gradual fall was observed in the group 3 rats (treated with 400 mg/kg body weight). Conversely, there was dose dependent progressive decline in the creatinine level of the rats.

Effect of Aqueous Extract of *S. globulifera* on Reproductive Hormones in the Male Rats

Repeated oral treatment with aqueous extract of *S. globulifera* resulted in significant ($p < 0.05$) increases in the sera FSH and LH levels when compared to the untreated control group (Table 5). The FSH dose-dependent increase was around 3 folds for group 4 rats (400 mg/kg body weight). However, testosterone and prolactin reduced significantly when compared to Group I values (Table 5).

Effect of Aqueous Extract of *S. globulifera* on Seminal Fluid Analytical Parameters in the Male Rats

Repeated daily oral treatment with 100 mg/kg/day, 200 mg/kg/day, and 400 mg/kg/day *S. globulifera* for 21 days on semen analysis showed significant ($p < 0.05$) dose-related increases in the total sperm count of treated rats with the most significant boost recorded in rats treated with the highest dose (400 mg/kg/day) of the extract which was comparable with what was recorded in the control (Table 6). The same pattern was recorded for % motile sperm counts. There was significant increase in sperm morphology when compared to the control untreated group. The highest increase was observed for group 2 rats (100 mg/kg body weight). (Table 6).

Effect of Aqueous Extract of *S. globulifera* on Testes in the Male Rats

In testes, histologic section shows seminiferous tubules lined by germ cells in various stages of development (the spermatogenic series), and containing luminal spermatozoa. Leydig cells are seen in the interstitium. Mild Reduction in lining cells of the seminiferous tubules, to about 3 cell layers thick. They are scanty (less compact). No luminal spermatozoa seen (Figure 1).

Phytochemical	Result
Tannin	++
Phlobatanins	nd
Saponin	++
Flavonoid	++
Steroid	++
Terpenoid	++
Cardiac Glycosides	++

Key: ++ =present nd = Not detected

Table 1: Phytochemical screening analysis of aqueous extract of *S. globulifera*

	Dose (mg/kg/day) body weight	Day1	Day 7	Day 14	Day 21
Group 1 (Control)	–	168.1 + 0.4	185.3 + 0.2	200.3 + 0.2	210.1 + 0.3
Group 2	100	172.0 + 0.1*	170.1 + 0.2*	164.1 + 0.03*	158.7 + 0.5*

Group 3	200	170.2 + 0.4	168.4 +0.4*	156. 1+0.4*	148.0 +0.1*
Group 4	400	172.4 +0.2*	164.0 +0.1*	150.2 +0.6*	142.3 +0.2*

Legend: Values represent Mean+ SEM of 6 rats and triplicate determination. *P < 0.05 significantly different from control

Table 2: Effects of aqueous extract of *S. globulifera* on the mean body weights of adult male wistar rats.

Group/ Treatment	Dose (mg/kg/day)	Liver(g)	Kidney(g)	Lungs(g)	Heart(g)	Testes(g)
Group 1 (Control)		14.2 + 1.2	3.1 + 0.02	2.2 + 0.02	1.1 + 0.02	4.4 + 0.1
Group 2	100	11.6 + 0.4	2.8 + 0.1*	2.6 + 0.2	1.3 + 0.06	3.8 + 0.3*
Group 3	200	12.0 + 1.4	3.0 + 1.6	2.3 + 0.2	1.2 + 1.3*	4.1 + 0.1*
Group 4	400	12.5 + 0.9	2.8 + 0.1	2.4 + 0.2	1.2 + 0.04	4.1 +0.03*

Legend: Values represent Mean + SEM of 5 rats and triplicate determination. *P < 0.05 significantly different from control

Table 3: Effects of aqueous extract of *S. globulifera* on the mean organ weights of adult male rats after 21 days of treatment

Group/ Treatment	Dose (mg/kg/day)	UREA (mmol/l)	URIC ACID (mmol/l)	CREATININE (mmol/l)
Group 1 (Control)	-	6.6+0.03	111.0+0.6	47.0+1.2
Group 2	100	5.7+0.1*	101.3+0.9*	40.7+1.5*
Group 3	200	9.5+0.3*	174.0+1.2*	37.0+0.6*
Group 4	400	5.7+0.1*	139.0+1.2*	40.6+0.9*

Values represent Mean+ SEM of 5 rats and triplicate determination. *P < 0.05 significantly different from control.

Table 4: Effects of aqueous extract of *S. globulifera* on renal function indices in adult male wistar rats.

Group/ Treatment	Dose (mg/kg/day)	Testosterone (ng/ml)	FSH (mlul/ml)	LH (mlul/ml)	PROLACTIN (ng/dl)
Group 1 (Control)	-	42.9+1.5	3.8+0.6	4.0+0.6	4.1+0.5
Group 2	100	18.6+1.1	5.3+1.1	4.10+0.5	0.8+0.1
Group 3	200	4.3+0.9*	5.1+0.4	4.0+0.3	0.8+0.1
Group 4	400	3.7+0.9*	10.1+0.1*	3.9+0.1	0.7+0.1

Values represent Mean+ SEM of 5 rats and triplicate determination. * P < 0.05 significantly different from control

Table 5: Effect of aqueous extract of *S. globulifera* on different fertility hormones of adult male wistar rats.

Group/ Treatment	Dose (mg/kg/day)	Sperm motility %	Sperm morphology	Spermcount X 10 ⁹
Group 1 (Control)	-	81.5+5.05	3.7+0.85	20.0+0.41
Group 2	100	72.5+6.51	10.25+0.85*	17.5+0.96

Group 3	200	85.5+5.42	8.25+1.11*	20.0+0.41
Group 4	400	85.5+5.42*	8.25+1.11	22.05+0.63*

Values represent Mean+ SEM of 5 rats and triplicate determination. * P < 0.05 significantly different from control.

Table 6: Effect of aqueous extract of *S. globulifera* on SFA of adult male wistar rats.

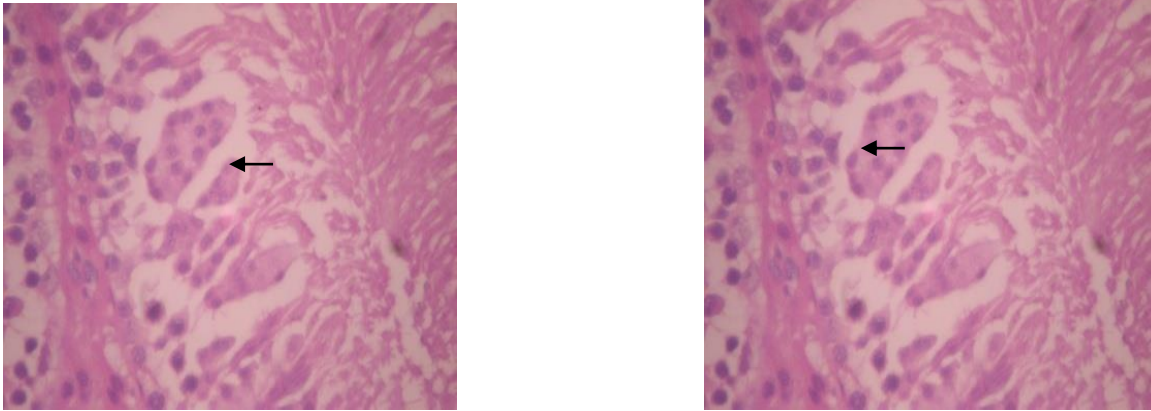


Figure 1: Photomicrographs of male wistar rats testes of (a) control untreated group 1 and (b) group 4 treated with 400 mg/kg body weight. The photomicrographs show seminiferous tubules lined by germ cells and containing luminal spermatozoa. Leydig cells are seen in the interstitium. Mild Reduction in lining cells of the seminiferous tubules, to about 3 cell layers thick. They are scanty and less compact (Black arrow). Mag X400.

Discussion

Medicinal plants have been used since the beginning of time to aid in many different ailments. Up to 80% of the world's population is either partially or wholly dependent on herbal products for the treatment of their different ailments [27]. One of these ailments is infertility which has been even corrected by the use of certain plants [28]. Infertility is the inability of a couple of opposite sex to achieve a clinical pregnancy after regular unprotected sexual intercourse. It is otherwise, a complete failure of a sexually competent and active couple to achieve pregnancy in one or more years despite regular sexual exposures [1]. There are several factors that has been attributed to infertility. These include nutritional factors, environmental factors, physiological factors, premature ejaculation, xenobiotics, malfunctioning testes, age, oxidative stress and genetic factors [2, 29]. Apart from orthodox treatment of infertility, the use traditional/complementary medicine as therapeutic alternatives for their infertility has been reported³⁰. Some of the plants with their extracts reported to enhance aphrodisiacs are *Eurycoma longifolia* [31], *Butea frondosa* [32], *Curculigo orchioides* [33], *Panax quinquefolius* (ginseng) [34] etc. and those that improved seminal fluid parameters are *Asparagus racemosus*, *Withania somnifera*, and *Andrographis paniculata* [35, 36].

We have accessed the fertility-enhancing property of *S. globulifera* in male Wistar rats in this investigation. Hormonal analysis of the treated rats revealed significant increase in the FSH and LH while it decreased circulating prolactin and testosterone. Serum elevation in the FSH and LH improves sperm quality in relation to sperm count, volume, motility, and morphology [33]. FSH and LH strongly influence spermatogenesis. They influence the fate of germ cells which is mediated by the actions on specific transmembrane receptors, Follicle Stimulating Hormone receptor (FSH-R), and Luteinizing Hormone receptor (LH-R) that are expressed in the Sertoli cells and interstitial Leydig cells, respectively [37]. In this study, oral treatment with 100, 200 and 400 mg/kg/day of *S. globulifera* stimulated significant spermatogenesis as a result of increased sperm count, sperm motility, and improved sperm morphology. This is in good agreement with the work of Adeneye et al [2]. In that work, it was reported that, water seed extract of *Hunteria umbellata* increased spermatogenesis

in male wistar rats. The increase in sperm count following the administration of the extract was further corroborated by the microphotograph seen in the testicular histology (Figure 1).

Also, the non-dose dependent significant increase in the serum levels of uric acid, and with non-significant effect on direct and total bilirubin are indications that there is no impaired hepatic secretory function (Table 4). Tissue creatinine is largely derived from endogenous source by tissue creatine breakdown and is also related to tissue mass. Significant depression of either urea or creatinine indicated that there is no impaired glomerular and impaired kidney function [38].

Conclusion

The oral administration of aqueous extract of *S. globulifera* increases FSH, and seminal fluid parameters. It therefore possess fertility enhancing effect on adult male Wistar rats since seminal indices are the main parameters for the assessment of fertility. Based on this result, this extract has the potentials of being developed into a male seminal and fertility enhancing agent.

Conflict of Interest

The authors declare no conflict of interest

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