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EVALUATION OF FERTILITY ENHANCING ACTIVITY OF AQUEOUS EXTRACT OF TERMINALIA CATTAPPA LEAVES AGAINST MALE ALBINO RATS

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ABSTRACT

Infertility is a disease of the male or female reproductive system defined by the failure to achieve pregnancy. Infertility affects millions of people of reproductive age worldwide – and has an impact. Estimates suggest that between 48 million couples and 186 million individuals live with infertility globally. In the male reproductive system, infertility is most commonly caused by problems in the ejection of semen, absence or low levels of sperm, or abnormal shape (morphology) and movement (motility) of the sperm. In the female reproductive system, infertility may be caused by a range of abnormalities of the ovaries, uterus, fallopian tubes, and endocrine system, among others. The number of pharmacological investigations on Terminalia catappa has been so far. The present study is to evaluate the fertility-enhancing activity of Terminalia catappa in experimental animals (MALE Albino rats).

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INTRODUCTION

Terminalia catappa is a large tropical tree in the Leadwood tree family, Combretaceae, native to Asia, Australia, the Pacific, Madagascar and Seychelles. Common names in English include country almond, Indian almond, Malabar almond, sea almond, tropical almond, beach almond and false kamani. The tree grows to 35 metres (115 feet) tall, with an upright, symmetrical crown and horizontal branches. The fruit is corky and light and dispersed by water. As the tree gets older, its crown becomes more flattened to form a spreading, vase shape. Its branches are distinctively arranged in tiers. The leaves are large, long and 10–14cm broad, ovoid, glossy dark green, and leathery. They are dry-season deciduous; before falling, they turn pinkish-reddish or yellow-brown, due to pigments such as violaxanthin, lutein, and zeaxanthin.The trees are monoecious, with distinct male and female flowers on the same tree. Both are 1 cm ($^{3}/_{8}$ in) in diameter, white to greenish, and inconspicuous with no petals; they are produced on axillary or terminal spikes. The fruit is a drupe 5–7 cm long and 3–5.5 cm broad, green at first, then yellow and finally red when ripe, containing a single seed. Pollen grains measure about 30 microns.The species epithet is based on its Malay name *Ketapang*.

The leaves obtained from the Terminalia cattappa have been used in traditional medicine for their anti-bacterial, hepatoprotective, hypoglycaemic, antioxidant, anti-inflammatory, and anti-microbial. In addition, the clinical use of leaves obtained from Terminalia cattappa is acting as ACE inhibitory activity and chemoprotectivethe present study is to evaluate the fertility-enhancing activity of Terminalia cattappa leaves in male Albino rats.

OBJECTIVES OF THE STUDY:

The objectives of the present study are

Several pharmacological investigations on Terminaliacatappahave been reported so far. However, there is a need to evaluate the fertility-enhancing activity of Terminalia catappa in experimental animals. The present study is, therefore, undertaken with the following objectives.

1. Extraction of Terminalia catappa with suitable solvents.
2. Preliminary phytochemical screening of crude extract.
3. Determination of LD50 on mice.
4. Evaluation of aqueous extract of Terminalia catappa for fertility fertility-enhancing

• Analysis of epididymis spermatozoa

i. Sperm motility and abnormality
ii. Sperm count
iii. Sperm viability

MATERIALS AND METHODS:

PLANT MATERIAL:

The leaves of the plant Terminalia catappa were collected from Narasaraopet. The plant herbarium specimen was identified and authenticated in the Department of Botany, Acharya Nagarjuna University, Guntur (522002) Andhra Pradesh

EXTRACTION:

The leaves of Terminalia catappa were dried in shade and powdered, the powder of theLeaves initialwas defeated with water (60-80°C) the extract was filtered using Whatman paper (No 1) and then concentrated in a vacuum and dried at 45°C for water eliminationand the extract was kept in a sterile bottle under refrigeration condition of about 2-8°C.

EXPERIMENTAL METHODS:

Acute (oral) Toxicity study (Fixed Dose Procedure) :

Method:

Acute toxicity studies for aqueous extract of Terminalia cattappa were conducted per OECD guideline 420 (modified, adopted) using Albino Wister mice. Each animal was administered aqueous an extract solution by oral route. The test procedure minimizes the number of animals required to estimate the oral acute toxicity of a chemical and in addition estimation of LD_{50}, confidence intervals. The test also allows the observation of signs of toxicity and can also be used to identify chemicals that are likely to have low toxicity.

Principle of the Fixed Dose Procedure.

The fixed procedure is a method for assessing acute oral toxicity that involvesthe identification of a dose level that cause evidence of non-lethal toxicity (termed evidenttoxicity) rather than a dose level that causes lethality. Evident toxicity is a term describingclear signs of toxicity following the administration of a test substance, such that an increase to thenext highest fixed dose would result in the development of severe toxic signs and probablymortality.

Procedure: As suggested, after the acclimatization of animals for 4-5 days, a study was carried outas follows:

- Healthy, young adult Albino Wistar mice (20-25gms), null porous and non-pregnant wereused for this study Food, but no water waswithheld for 3-4 hours and further 1-2 hourspost administration of sample under study.
- Fixed levels level of 5, and 50, 500 mg/kg were initially chosen as levels that would beexpected to allow the identifications-producing using evident toxicity.
- During the validation procedure, a fixed dose of 2000mg/kg was added to provide moreinformation on substances of low acute toxicity.
- Dosed one animal at the test dose by oral route.
- Since, this first test animal survived, four other animals were dosed (orally) in subsequentdays, at a total of five animals were tested.

Observation:

Animals were observed individually at least every 5 minutes once during the first 30minutes after dosing, periodically at 2 hrs during the first 24 hours (with special attentionduring the first four hours) and daily thereafter, for a total of 14 days.

Fertility studies:

Experimental design

The male rats were divided into 3 groups consisting of 5 animals.
Group I: Rats received normal saline daily for 14 days, orally (Normal control).
Group II: Rats received an aqueous extract of leaves of Terminalia catappa at a dose of 250Mg/Kg body weight daily for 14 days.
Group III: Rats received an aqueous extract of leaves of Terminalia catappa at the dose of500 mg/Kg body weight daily for 14 days.
After 24 hours of the last treatment, the final weight was recorded and the animals weresacrificed by decapitation.

Sperm count:

Epididymal fluid (for sperm count) was collected from caput and cauda segments separatelyand diluted with Sorenson's buffer (pH 7.2). The separated fluid was taken for sperm count. Sperm count was carried out by using Neubauer's haemocytometer

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 themicroscope slide and 2 drops of normal saline were added to mobilize the sperm cells.Epididymis sperm motility was then assessed by calculating motile spermatozoa per unitarea. Morphology (abnormality) was evaluated on sperm from the caudal epididymis. Thetotal morphological abnormalities were observed.

SPERMVIABILITY:

When semen motility is examined some of the sperms are immotile. It is difficult toknow whether such sperms are alive or dead. To know the vitality of a vital stain is necessary.Eosin is used to differentiate live and dead sperms where the dead sperms take the Eosin stainand live sperms do not take the stain. Eosin can penetrate dead spermand take the stain. Eosincan penetrate dead sperms and in live sperms, it cannot. This is taken as a criterion todifferentiate between live and dead sperm cells (Fd M et al., 1965).

Preparation of the stain:

Eosin Y – 0.5 gms
Distilled water – 100ml
Mix well and filter through filter paper.
A. Nigrosin water soluble-10 gms
Distilled water-100ml
Dissolve Nigrosin thoroughly under warming and filter.
Preserve both stains in the refrigerator.

Procedure:

- Mix 25µl of semen with one drop of 0.5 % Eosin solution in a microcentrifugeand wait for 10 seconds.
- To the above mixture add three drops of 10% Nigrosine stain and mix gently.
- From the above mixture make thin smears using another clean-edged glass slide.
- Dry them in the air and examine the sperms under oil immersion.
- Count 100 no. of sperms using a 40 X magnification lens in a compound microscope.

Classify the different abnormalities in percentage.

RESULTS:

The percentage yield of AETC:

The shade-dried leaves of Terminalia catappa. Weighing about 150g was extracted fromthe hotmaceration process. The weight of the aqueous extract obtained was 14.00g. Its percentage yieldis calculated by the following formula:

$$\textbf{Percentage yield:} \quad \frac{\textbf{Weight of the extract obtained*100}}{\textbf{Weight of Crude powder}}$$

The percentage yield of the aqueous extract of Terminalia catappa (AETC) is 9.33% w/w.

Preliminary Phytochemical Investigation:

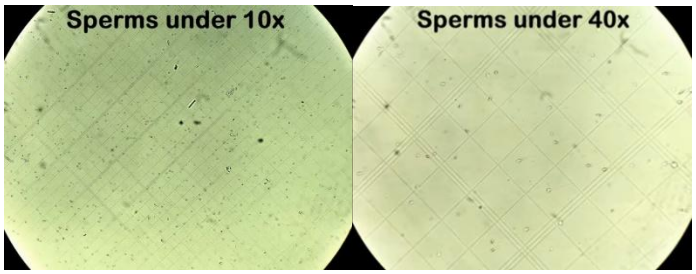
The results of the preliminary phytochemical screening of the aqueous extract ofleaves of Terminaliacatalpa. were shown in the Table. The aqueous extract gave positiveresults for, reducing sugars, phenols, tannins, flavonoids, and triterpenoids.

Preliminary Phytochemical Screening for AETC

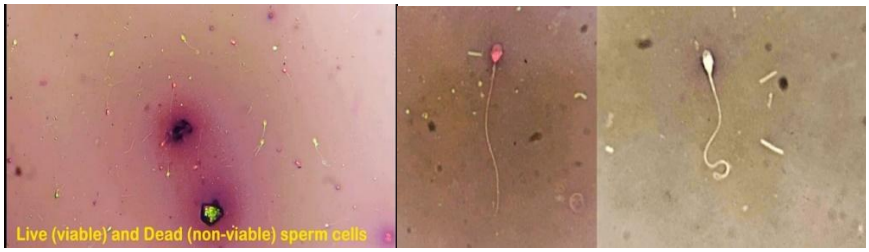
S:No	Phytochemical Tests	Results
1	Alkaloids	Absent
2	Reducing sugars	Absent
3	Saponins	Absent
4	Tannins	Present
5	Flavanoids	Present
6	Steroids	Absent
7	Terpenoids	Present

EFFECTS OF AQUEOUS EXTRACT OF TERMINALIA CATTAPPA LEAVES ON THE SPERM CONCENTRATION AND MOTILITY IN THE EPIDIDYMIS OF ADULT MALE ALBINO RATS

GROUPS	SPERM COUNT(10^6ml)	SPERM MOTILITY (%)	SPERM VIABILITY (%)
CONTROL	55	69	71
TEST-1 (250mg/kg)	63	89	63
TEST-2 (500mg/kg)	75	119	26

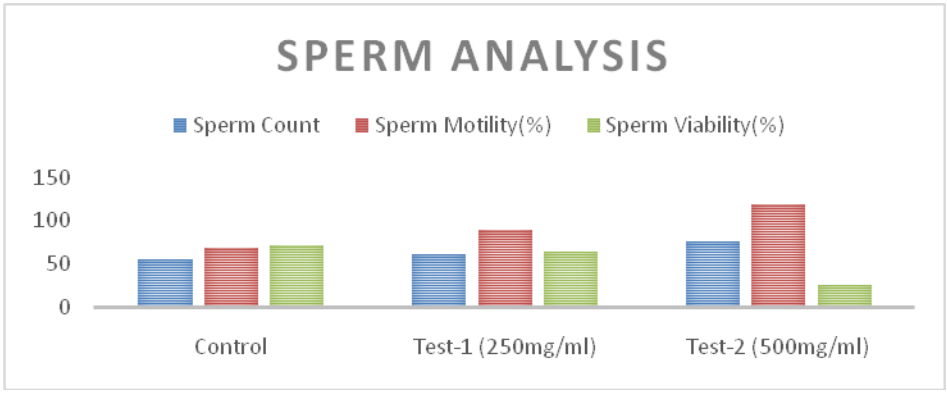


Sperm count



Sperm Viability

Sperm Motility **Sperm Abnormality** **Sperm Morphology**



DISCUSSION

Around the world, one out of six couples has difficulty conceiving. Infertility is defined as one year of regular and unprotected intercourse without conception. On evaluation, roughly 50% of affected couples have causal or associated male factors as a cause of infertility. Couples suffering from infertility use concomitantly traditional medicine from natural plants and modern medicine as possibilities of treatment. The use of medicinal plants in the treatment of diseases and dysfunctions goes back several millennia and has considerably contributed to the development of pharmaceuticals since about 25% of modern drugs are derived from plants. In addition, up to 60% of the world's population uses herbal products for medical purposes. For two decades, the evaluation of natural materials as a source of potential drugs has been of resurgent interest in developing countries as well as in developed ones. This growing interest inphytotherapy is due to several reasons namely; conventional medicine can be inefficient (ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems, finding of the "natural", the large therapeutic spectrum of plant products and their effectiveness in the treatment of chronic diseases, need for the development of new drugs. Bioflavonoids were first discovered by Nobel Prize laureate Albert Szent Gyorgyi in the year 1930. Flavonoids are a ubiquitous group of polyphenolic compounds of variable chemical structures present in fruits, vegetables, nuts, seeds and beverages such as tea, coffee, beer and wine28. More than 4000 types of flavonoid compounds have been isolated from various plants. Bioflavonoids are having antiviral, anti-bacterial and anti-fungal activities also. The structure of bioflavonoids is characterized by a C6-C3-C6 skeleton.
Flavonoids occur as aglycone without sugar moiety and a glycoside.

Aglycone	Glycoside
Hesperidine	Hesperitin
Diosmetin	Diosmin
Qercetin	Rutin
Naringenin	Naringin

Depending on their structural features, flavonoids can be further subdivided intoflavones, flavonols, isoflavones, flavanes and flavanols". Some were found to possessanti-inflammatory" antiallergicantiplatelet", antineoplastic,anti-ischemicantilipoperoxidant", gastro-protective properties and other effects have also been described.It is suggested that most of these biological effects are related to their antioxidant activity.Flavonoids can exert their antioxidant activity by various mechanisms, e.g. by scavengingor quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for the generation of free radicals The Terminaliacatappa for fertility-enhancing activity in sperm parameters taken upfor the study. The results reveal that aqueous extract at 500mg/kg had shown an effectsignificantly when compared to the control as evidenced by the increase in sperm count and spermmotility and a decrease in sperm viability. The above discussion shows that the Terminaliacatappa is said to produce beneficial fertility-enhancing activity,Review of the available literature revealed that the majority of the fertility-enhancing takes place by different constituents like flavonoids, tannins, which have been reported to be responsiblefor the fertility-enhancing activity. From the phytochemical studies, this plant is said to possess active tannins flavonoids this was supported by the fact that the Terminalia catappa is used widely for fertility-enhancing activity in folk medicine.

CONCLUSION

The present study was an attempt to evaluate aqueous extracts of the Terminalia catappa for fertility-enhancing activity.Fresh leaves were collected, shade dried, powdered, and extracted with water. PhytochemicalStudies of the extracts were carried out and extracts showed the presence of tannins and flavonoids.The acute toxicity studies of Terminalia catappa aqueous extracts were conducted asPer OECD guidelines 420. Acute toxicity studies have revealed that the aqueous extracts ofTerminalia catappa were safe up to 2000 mg/kg.The fertility-enhancing activity of aqueous extracts of Terminalia catappa was screenedfor sperm parameters. The extracts have produced fertility-enhancing activity as evidenced bythe results obtained from the studies.The interpretation of the results was done after subjecting the data obtained from variousstudies toa chart. The studies indicate that the aqueous extracts of Terminalia catappa producea promising increase in fertility.

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