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Research Paper

Measurement the Antifertility Potential in Male Rats Exposed to The Electromagnetic Signals Emitted by Cellular Mobile Telephony

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Abstract

The present study was conducted to measure the antifertility potential of *Curcuma amada* in male rats exposed to the electromagnetic signals emitted by cellular mobile telephony, which can affect their biological functions, behaviors, and multiple hormone systems leading to reproductive deficit. In addition, oxidative stress, calcium deficiency and disturbances in signal transduction and regulation pathway can be emerged by the alternation of spermatozoa. It has been revealed that Medicinal plants, also called medicinal herbs are more productive than synthetic drugs. Accordingly, in this work we showed that the pretreatment of *Curcuma amada* can substantially improve the sperm count, and motility. It is also able to decrease the cell phone radiation induced infertility in rats. The extract has shown significantly potential impact on the Testosterone hormone levels, indicating the protective effects against radiation-induced infertility.

Keywords: *Curcuma Amada*; Infertility; Spermatozoa; Cell Phone Radiation; Rat

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1. Introduction

One-in-six couples have fertility problem in the word, and it is defined as a one-year regular unprotected intercourse without any conception. In a study, approximately 50% of affected couples show causal or related male factors as a main root of infertility. Those who are suffering from infertility problems, permanently use of different traditional medicine from natural plants and modern ones as a possible cure has been recently suggested. The use of medicinal plants in the treatment of diseases and dysfunctions goes back to several millennia, and has considerably contributed to the development of pharmaceuticals since about 25% of modern drugs are derived from plants. Using of plants as remedy of different disorders, goes back to our ancestors' times and since then, has played a potential role in the development of pharmaceuticals during which many scientists estimated that near 25% of modern drugs are derived from traditional plants. Besides, to medical attention, more than 60% of the world's population are using herbal products, and from 20 years ago, the growing use of natural plants as the one of the most important sources of drugs has been seen vastly in the both developed and devolving countries. This interest, which is growing for Phytotherapy every year, is supposed to be because of a couple of reasons including; conventional medicine can be insufficient now. In fact, abusive and incorrect use of synthetic drugs results in further defensive side effects. On the other hand, finding the "natural", large therapeutic spectrum of natural plant products and their effectiveness in the cure of chronic diseases, the need for the development of new drugs. Phytomedicines are dietary supplements with nutritious and refreshing

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effects on the different organisms. People are also highly encouraged by the World Health Organization for using medical plants, and many researchers are invited to do their best to identify the logical uses of medical plants as drugs.

The beneficial ingredients extracted from these herbal plants are today largely utilized to treat or act as palliative for male various infertility such as: lack of libido, sexual asthenia, erectile dysfunction, ejaculatory and relaxation dysfunctions, loss of orgasm, and sperm abnormalities. Despite the fact that the causes of male infertility are diverse, it has been shown that psychogenic and endocrine disorders, vascular damages and drug abuse can be as symptoms. Accumulating evidence from *in vitro, in vivo* and clinical surveys proved the empirical use of plants in the treatment of male fertility parameters. This review article was therefore undertaken to evaluate the potential use of medicinal plants in the treatment of male infertility symptoms in rats, including; libido dysfunction, erectile and ejaculatory disorders, and sperm abnormalities, and the possible effects of phytomedicines as the mode of treatment.

In fact, infertility refers to the inability of a person to produce an offspring, and it believes to be a medical and social preoccupation. It has become globally a big issue and can be demarcated as the loss of pregnancy within 12 months or more of constantly unprotected intercourse. It has been proved that more than 15 or 20 per cent of mates in the reproductive ages are infertile, ascribing to on both male and female sides and almost 30% of infertilities are considered to be on the male side. Infertility of women voices the concerns globally; however, the most important cause is reported as lower sperm count. Over 5 decades, the investigations in the western countries have proved indicated that sperm numbers have dropped by 1 % each year, and the estimation of the numbers of sperm, their mobility and other morphological features has shown the significant decrease compare to past in the infertile men, while under feeding men shown the adverse effects.

Doing animal experiments, it has been demonstrated that delay in sexual maturity which causes swift regressive changes in male organs happens with the restriction of nutrient intake or lack of specific nutrients. Thus, a simultaneously strong and successful reproduction requires complete ingredients of macro-and micronutrients containing; iron, calcium, minerals, phosphorous, carbohydrate, fibers, Phenolic compounds such as

Calcium-Initiates sperm motility.

Phosphorous – it forms the sugar-phosphate back bone of DNA and RNA.

2. Materials and Methods

2.1 Plant materials and extraction

Fresh and healthy *Curcuma Amada* rhizomes of 3 kg were collected from the local trader and then shade dried. The rhizomes of *curcuma Amada*, which is popularly known as mango-ginger were authenticated and certified by Dr. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur.

The dried plant has been powder (300g) at a mixing ratio of 1:4 (w/v) mango ginger powder: the solvent was added to stirred solution of 50% v/v aqueous ethanol (1200mL) at 40°C. The resulting mixture was stirred over 30 min and then to be cold, we put it in the room temperature. Finally, the mixture was centrifuged during 7minutes and the supernatant was drawn off. The pellet was resuspended in fresh solvent and re-centrifuged. The supernatant was filtered, and hydro alcoholic extract so obtained was dried and powdered.

2.2 Acute toxicity

Based on the OECD guidelines, page 423, the possible acute toxicity of the hydroalcoholic extract of *Curcuma amada* was analyzed for 10 rats in various groups, and they had been administrated based on every kg body weight in different doses of 100, 200, 300, 400, 500 and 1000 mg/kg. The number of deaths and behavioral changes were evaluated and recorded within 48h. We did not have any mortality or toxicity for the up to1000mg/kg rats. Based on the previous studies, 200 and 300mg/kg body weight of *Curcuma amada* were selected for the current experimental study.

2.3 Animal model used

We used the normal animals, which had free access to food and water, and all groups received the plant extract alone (200 mg/kg). The minimum dose was 100 mg/kg daily and the maximum was 300 mg/kg.

2.4 Exposure to mobile phone radiation:

for 28 days, the animal was exposed 2 hours for a day. Exposure took place in a ventilated Plexiglas cage and kept in an anechoic chamber in the far-field approximation for a mobile phone. The male rats were irradiated at the same time between 50 and 100 hours, the caged animals then were removed one by one and weighed. They were allowed to easily move into the exposure box through the rear entrance, aluminum foil was used for sealing the box, and then they carried into the adjacent exposure facility, and pre-aligned chocks on top of a pedestal was given to the rats.

2.5 Surgical procedure

having finished the treatment days, the rats were first weighed and then sacrificed by cervical dislocation. To expose the reproductive organs, the abdominal cavity was opened up via a midline abdominal incision. The testis was detached and cleared free of the surrounding tissues.

2.6 Semen analysis

2.6.1 Sperm count

Haemocytometer was used for the total sperm count, which is used for both RBC WBC counting, and It is included; the pipettes for the dilution of the blood samples and a special type of ruling called Neaubaur's slide. Ruled squares on the slide would be utilized for counting. In our survey, the epididymis was removed and transferred in a pre-chilled Petri-plate and 2mL of 0.9% saline was added in a way that the epididymis could be gently minced with the help of sharp razor. This sample was used for the sperm count, and then it was a pipette out with the help of pipette provided in the Haemocytometer. A clean and dry coverslip was kept on the Neaubaur's ruling.

By touching the tip of the pipette to the slide, the ruling was loaded with the sample. To allow the sperms to settle down, for 2 minutes the slide was kept on a bench. The sperm counting was begun and they were counted in four squares at the corner of the ruling, which is covering an area of 4sq.mm. under high power objective. The accurate counting of spermatozoa with head and tail was done.

The counting was based on this formula; Sperm number = average number of spermatozoa counted x multiplication factor (10'000)

Dilutionfactor (20) = N x10'000 x20 = N x0:2x106 spermatozoa.

Table 1. The effect of *curcuma amada* on sperm count and sperm motility

Treatment	Sperm count	Sperm motility
Normal (saline)	41.67±1.07	52.50±2.51
Extract Alone(200mg/kg)	45.00±1.526	52.12±2.750
Diseased	26.67±3.33	34.00±3.73
High Dose (300mg/kg)	54.17±2.356	64.50±1.60
Low Dose (100mg/kg)	27.50±2.141	30.00±1.82

2.6.2 Sperm motility

A drop of sperm suspension was transferred to a glass slide, which was clear and then it was covered by a cover slip. The slide was then analyzed under the microscope at 400X magnificent, and the sperm motility and viability was scored in different points of view. To consider their movements, spermatozoa was evaluated. The whole spermatozoa consist of motile and immotile as well were counted with the accompaniment of a blood cell count. Based on the following formula the sperm motility was calculated:

Motility%=number of motile spermatozoa / total number of spermatozoa (motile +immotile) X 100

2.6.3 Hormonal assays

In order to do the hormonal assays test, after collecting the rats' blood by cardiac puncture under anesthesia, they were centrifuged, the serum was collected and stored. By Chemiluminescence immune assay using immunoassay kits and the ADVIA Centaur immune analyzer, the serum testosterone was assessed.

2.6.4 Serum testosterone

Testosterone assay is a competitive immune assay using direct chemiluminescent technology. Testosterone in the sample competes with acridiniumester-labeled testosterone in the Lite Reagent for a limited amount of polyclonal rabbit anti-testosterone anti body bound to monoclonal mouse anti- rabbit antibody, which is coupled to paramagnetic particles in the Solid Phase. The assay uses Testosterone Releasing Agent to release the bound testosterone from the endogenous binding proteins in the sample. Results were expressed as mg/dL.

3. Results

All the Values are means ± SEM, n = 5 per treatment group and are significant when analysed by one-way ANOVA with Tukey's post hoc test. 'a'p<0.05; 'c'p<0.001when compared to normal control; p<0.001 when compared to disease control.

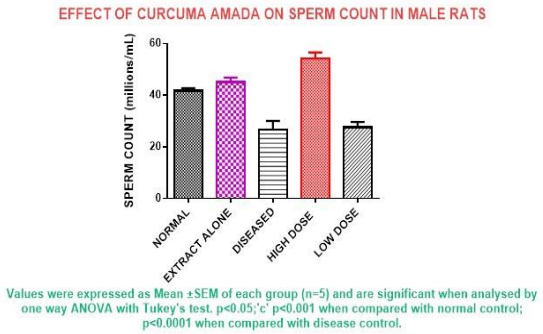

Figure 1. The effect of *curcuma amada* on sperm count in male rats

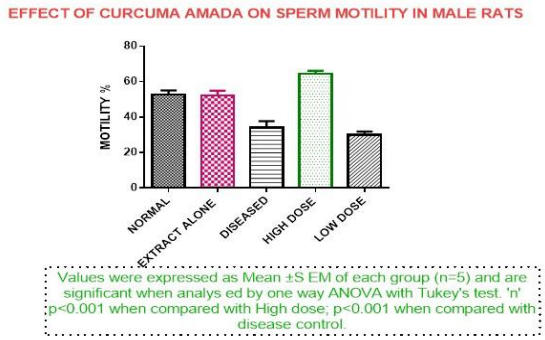

Figure 2. The effect of *curcuma amada* on sperm motility in male rats

Table 2. The effect of *curcuma amada* on testosterone hormone

Treatments	Testosterone (ng/dl)
Normal (saline)	1.203±0.11
Extract alone(200mg/kg)	1.42±0.35
Diseased	0.34±0.09
High dose (300mg/kg)	3.22±0.38
Low dose (100mg/kg)	0.673±0.204

4. Discussion

Fertility is considered as the natural capability of a couple to produce offspring during a year of unprotected sexual intercourse. As the indices of fertility in men, the normal morphological attributes, total sperm count and also motility have been described.

Spermatogenesis is a precise process occurring in a series of highly organized steps through which normal diploid cells finally transformed into the sperm, happening in the seminiferous tubules that is the place of sperm cell formation and maturation and the principal section of testes.

The fundamental markers in testicular spermatogenesis and epididymal maturation, which supposed to be the critical indices of male fertility are sperm parameters such as count and motility.

Sperm parameters such as count and motility are key indices of male fertility, as these are the prime markers in testicular spermatogenesis and epididymal maturation. In addition, it has been shown that for the development, growth, and normal functioning of the testes and male accessory reproductive glands, androgens would be imperative.

Gonadotropins as the gonads which are essential for commencement and maintenance of spermatogenesis in male rats can result in the secretion of testosterone in males. To maintain the structure and function of the male accessory sex glands, testosterone is essential; therefore, lack of testosterone disrupts spermatogenesis.

It has been approved that radiofrequency electromagnetic fields (RF-EMF) can have an opposite effect on the quality human sperm that can effect on fertilization potential.

This survey evaluated the effect of RF-EMF on sperm-specific characteristics to assess the fertilizing competence of sperm. Highly motile human spermatozoa were exposed for 1 hour to 900-MHz mobile phone radiation at a specific absorption rate of 2.0 W/kg and tested for the various times after exposure. By flow cytometer, the acrosome reaction was evaluated and it shows that the radiation did not affect sperm propensity for the acrosome reaction.

Pretreatment with *Curcuma Amada* (100mg/kg, 200mg/kg and 300 mg/kg per day) substantially ameliorated the sperm count and motility, it also declined the effect of cell phone radiation which was induced infertility risk in male rats. Moreover, the extract has shown potential efficacy on the hormone systems which prove the protective effect of this herbal plant against infertility created by radiation-induced.

Moreover, the mango ginger rhizome is found to be a rich source of fibers and starch, and the antioxidant activity because of existing of different powerful antioxidants like beta carotenoids and several minerals like calcium etc. was reported. In the present study, *Curcuma amada* has shown significant powerful effect in the neutralization of radiation induced infertility in male rats. Chemical constituents' steroidal saponins, alkaloids and flavonoids are the main components of *Curcuma amada*. Steroidal saponins raise the testosterone level, and boost the sexual behaviors in rats. Flavonoids which are anti-aging can raise the dehydropepiandrosterone. Alkaloids would increase the release of NO from endothelia and nerve endings of male rats, dilate the blood vessels in male rats' sexual organs, and promote the sexual behaviors.

Based on the results that achieved in this study, it can be concluded that the attendance of these beneficial compounds in *Curcuma amada* extracts might be responsible for the protective role in infertility in male rats which was induced by radiation.

Treatment of these rats exposed to the mobile radiation resulted in the significant reduction of sperm count and motility, while the significant increase in the level of hormone serum testosterone was observed.

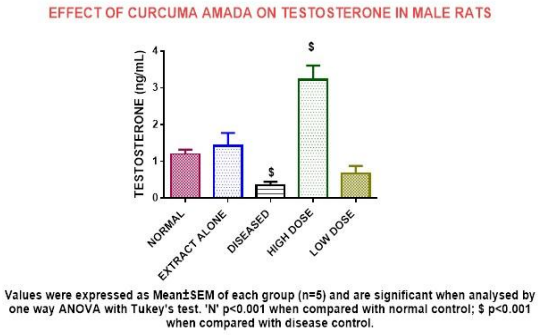

Figure 3. The effect of *curcuma amada* on testosterone in male rats

5. Conclusion

In the present study, pre-treatment of rats with *Curcuma Amada* (100, 200mg and 300mg/kg per day) illustrated that 100mg/kg body weight without radiation extraction only improved the sperm count, and the motility was also declined though the hormone levels were significantly increased.

The *Curcuma Amada* extraction shows powerful antioxidants such as curcumenoids, beta-carotenoids, and minerals like calcium and

phosphorus, and also it appears Saponins, glycosides and alkaloids.

In this study the obtained results and reports show; it can be concluded that the existing compounds in the *Curcuma Amada* extract might be responsible for the protective role against the mobile phone radiation which can cause the fertility in rats

Based on the data, reports and results in this study, it can be concluded that *Curcuma Amada* has the protective impact on mobile radiation which can cause the fertility in rats.

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