RESEARCH ARTICLE

Preclinical evaluation of spermatogenesis activity of mango ginger and GSM mobile phones radiation on the LH and testosterone hormones

Devarinti R

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BACKGROUND: Spermatogenesis is a complex process involving mitotic cell division, meiosis and the process of spermatogenesis. The regulation of spermatogenesis involves both endocrine and paracrine mechanisms. The endocrine stimulation of spermatogenesis involves both testosterone and luteinizing hormone, the latter acting through the intermediary testosterone, produced by the Leydig cells in the testis.
INTRODUCTION: Radiations are in energy form that's travelling through space in the form of waves. Cell phone radiation classified as a non-ionizing. Non-ionizing radiation refers to a variety of electromagnetic radiation.
HORMONAL ASSAYS: Testosterone Assay and Luteinizing Hormone assay.
RESULTS: Post-treatment with *Curcuma amada* 100 mg/kg, 200 mg/kg and 300 mg/kg body weight) significantly improved the levels of LH and testosterone hormonal levels. In the Radiation alone groups were observed the Decreases the above hormonal levels.
The present study was conducted to evaluate the protective effect of *Curcuma amada* on male reproductive system of hormonal estimations like total testosterone and LH against mobile phone radiation induced infertility in male Wister albino rats.
Key Words: *Spermatogenesis; LH; FSH; Curcuma amada; Rats*

INTRODUCTION

Radiations are in energy form that's traveling through space in the form of waves. The world health organization (WHO) announced May 31, 2011, that mobile phone emits radiation to it to the same carcinogenic list as exhaust from engines and lead [1-4]. Cell phone radiation classified as a non-ionizing. Non- ionizing radiation refers to a variety of electromagnetic radiation. Other forms of non- ionizing radiation include microwave, radio waves and visible light. Most of us cannot avoid mobile phone radiation. Around the world, one out of six couples trying to conceive has difficulties. Infertility is defined as one year of regular and unprotected intercourse without conception. On evaluation, roughly 50 % of affected couples have causal or associated malefactors as a cause of infertility.

In today's society, the modern man strives to become increasingly efficient. Our fast pace lives have been the driving forces behind vast technological innovations such as the Internet, email, and most recently, the "Smartphone". Cell phones have become a vital part of our lives, and as the social pressures for optimal efficiency increase, so do the technological capabilities of cell phones. The increase in popularity of cell phones is accompanied by a growing concern regarding the harmful effect of cell phone radiation (radiofrequency electromagnetic waves) RF-EMW exposure on human health [5-7].

Spermatogenesis is a process of involving mitotic cell division, meiosis. The regulation of spermatogenesis involves both endocrine and paracrine (local hormone diffuse a short distance to other cells). The endocrine stimulation of spermatogenesis involves the both follicle stimulating hormone and luteinizing hormone, the latter acting through intermediate testosterone; it takes about 70 days for sperm to become mature and able to fertilize an egg produced by the Lyedig cells in the testis. The thyroid gland is one of the most exposed and vital organs and may be a target for any type of electromagnetic radiation.

Cell phones emit radiofrequency electromagnetic waves (RF-EMW) to nearby relay base stations or antennas. Our bodies act as antennas that absorb the radiation and convert it into alternating eddy currents. The frequencies of these radio waves fall in the low-frequency microwave range (800- 2200 MHz). Therefore, this radiation is of non-ionizing type as the energy emitted is too low to break chemical bonds in a biological system. Cell Radiation is electromagnetic in nature, i.e., it consists of waves of electric and magnetic energy moving together through space at the speed of light. We live in a radiation world and are exposed to both natural and man-made radiation. Every second of our life, we are exposed to all forms of radiation such as ultraviolet light from the sun and radio waves from radio and radiofrequency electromagnetic waves (RF-EMW) from cell phones. Several recent studies have indicated that radiofrequency electromagnetic fields (RF-EMF) have an adverse effect on human sperm quality, which could translate into an effect on fertilization potential. This study evaluated the effect of RF-EMF on sperm-specific characteristics to assess the activity of sexual Hormones like Testosterone, FSH and Luteinizing Hormone. Were exposed for 1 h to 900-MHz mobile phone radiation at a specific absorption rate and examined at various times after exposure. Plants are a good source of a natural preparation containing effective bioactive constituents which can be used for different applications particularly health-promoting ingredients in the formulation of functional foods and nutraceuticals.

Review on *Curcuma amada* rhizome

Botanical name: *Curcuma amada* Roxb.

Family: Zingiberaceae.

Curcuma amada is a unique spice having morphological resemblance with ginger but imparts a raw mango (Mangiferaindica) flavor. The genus name *Curcuma* was coined by Linnaeus in 1753 in his Species Plantarum. The word probably derives from the Arabic word ‘kurkum', which means yellow color. *Curcuma amada* Roxb is commonly known as Mango Ginger.

Ayurveda and Unani medicinal systems have given much importance to mango ginger as an appetizer, aphrodisiac and antipyretic. The biological activities of mango ginger include antioxidant activity, antibacterial

Department of Clinical Research, KLE University, Karnataka, India.

Correspondence: Devarinti R, Clinical Research Department, KLE University, Karnataka, India.
Telephone +918886042417, e-mail siddu3pharma@gmail.com

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activity, anti-inflammatory activity, platelet aggregation inhibitory activity, and cytotoxicity.

The major chemical components include Starch, Phenolic acids, volatile oils, curcuminoids, and terpenoids. This article brings to light the major active components present in *C. amadaa* Roxb with their biological activities. Based on this article confirmed the plant useful in the spermatogenesis (aphrodisiac activity).

The couples suffering from infertility use concomitantly traditional medicine from natural plants and modern medicine as possibilities of treatment [8-10]. 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 medicinal plants. In addition, up to 60 % of the world's population uses herbal products for medical purposes.

MATERIALS AND METHODS

Plant materials and extraction: *Curcuma amada* rhizomes of 3 kg were collected from the local trader and shade dried. The rhizomes of *Curcuma amada* (mango ginger) were authenticated and certified by the Department of Botany, Acharya Nagarjuna University, and Guntur.

Curcuma amada powder (300 g) at a mixing ratio of 1:4 (w/v) mango ginger powder: solvent was added to a stirred solution of 50 % v/v aqueous ethanol (1200 mL) at 40°C. The resulting mixture was stirred for 30 min and then cooled to room temperature. And then the mixture was centrifuged for 7 min and the supernatant was drawn off. The pellet was resuspended in fresh solvent and re-centrifuged. The supernatant was filtered, and hydroalcoholic extract so obtained.

A weight of the *C. amada* taken: 3000 gm (3 Kg).

Dried weight of *Curcuma amada*: 2800 gm.

The weight of the powdered *C. amada*: 2650 gm.

A weight of powdered *C. amada* taken for extraction: 300 gm.

No of extractions: 5.

The total yield obtained: 30 gm.

% yield: 1.9 %.

Exposure of mobile radiation

Animals were exposed for 2 h a day for 28 days. Exposure took place in a ventilated Plexiglas cage and kept in the anechoic chamber in a far field configuration from the mobile phones. Animals were irradiated one at a time between 50 and 100 hrs., the animal was removed from its cage, weighed, and allowed to move into the exposure box through the rear entrance, the box was then sealed with aluminum foil, carried into the adjacent exposure facility, and placed in pre-aligned chocks on top of a pedestal [11].

Mechanism of free radicals formation

Reactive oxygen species are free radicals, which plays a major role in the mechanism of the biological effects induced by electromagnetic radiation. In aerobic cells, reactive oxygen species (ROS) are generated as a by-product of normal mitochondrial activity. If not properly controlled, ROS can cause severe damage to cellular macromolecules, especially DNA [12]. There is a linear correlation between the overproduction of ROS and DNA damage induced by electromagnetic radiation. There is a building bridge of a possible mechanism between the free radical formation and cell function. It defines that electromagnetic field induces changes during an apoptotic process in cells due to oxidative stress of Oxygen reactive species. The radiofrequency field, acting, especially on Ca^{2+} ions. This change in Ca^{2+} results in the release of cytochrome C from mitochondria activation of caspases 9 and, consequently, of the effector caspases 3, 6 and 7 and, finally cell death through apoptosis.

Acute toxicity

As per the OECD guidelines 423, the acute toxicity of the hydroalcoholic extract of *Curcuma amada* was tested on different groups of 10 Rats. In this, each rat received different doses of 100, 200, 300, 400, 500 and 1000 mg/kg body weight. The number of deaths and behavioral changes were observed in each group and recorded within 48 h (Table 1). Upto 1000 mg/kg, there were no signs of toxicity and mortality [13,14]. Based on these studies 100, 200 and 300 mg/kg body weight of *Curcuma amada* was selected for the present experimental study.

Animals grouping:

Normal Control.

Extract alone CA (200 mg/kg).

Diseased (Radiation only).

High dose CA (300 mg/kg).

Low dose CA (100 mg/kg).

Table 1: Animal grouping and treatment of animals.

Sl.No	Group code	Description	Treatment of animals-mg/Kg
1	N	Normal	Nil
2	EA	Extract alone	200 mg
3	D	Diseased	Radiation
4	LD CA	Low dose *Curcuma amada*	Radiation 100 mg
5	HD CA	High dose *Curcuma amada*	300 mg

Allotment of animals

Male albino rats were divided into 5 groups of 6 rats each. Allotment of animals into groups was done by computerized randomization method (Table 2).

Table 2: Animal groups by computerized randomization method.

S.No	Group Code	Randomization Method					
1	N	3	11	22	17	7	2
2	EA	29	9	10	15	25	13
3	D	19	5	18	21	12	16
4	LD CA	8	24	30	26	20	4
5	HD CA	1	14	23	6	28	27

Collection of blood samples

Before the sacrifice of animals, the blood was collected from the animals by cardiac puncture under chloroform anesthesia and blood stored (2-8°C) for hormonal assays.

Hormonal assays

The blood was collected from rats by cardiac puncture under anesthesia, centrifuged and serum was collected and stored for hormonal assays. Serum testosterone, luteinizing hormone, follicle stimulating hormone were assessed by Chemiluminescencet immune assay using immune assay kits and ADVIA Centaur immune analyzer.

Testosterone assay

Principle: Testosterone assay is a competitive immunoassay using direct chemiluminescent technology. Testosterone in the sample competes with acridinium ester-labeled testosterone in the Lite Reagent for a limited amount of polyclonal rabbit anti-testosterone antibody 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 bound testosterone from the endogenous binding proteins in the sample [15] Results were expressed as ng/dL.

Luteinizing hormone assay

Overview on LH: Luteinizing hormone is hormone secreted/produced by gonadotropic cells in the anterior pituitary gland. In females: an acute rise of LH triggers ovulation and development of the corpus luteum. In male: LH also called intestinal cell-stimulating hormone (ICSH). It stimulates Leydig cell production of testosterone. It acts synergistically with FSH.

Principle: LH assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two antibodies that have specificity for the beta subunit of the intact LH molecule. The first antibody, in the Lite Reagent, is a monoclonal mouse anti-LH antibody labeled with acridinium ester [16-18]. The second antibody, in the Solid Phase, is a monoclonal mouse anti-LH antibody, which is covalently coupled to paramagnetic particles (Labs-Chemistry, n.d.). Results were expressed as mIU/mL.

Procedure: The system automatically performs the following steps:

50 µL of sample were dispensed into a cuvette.

100 µL of Lite Reagent was incubated for 5 minutes at 37°C.

400 Ml of solid phase was incubated for 2.5 minutes at 37°C.

A direct relationship exists between the amount of LH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

After separation, aspiration and washing the cuvettes with reagent water, 300 µL each of acid reagent and base reagent were dispensed to initiates the chemiluminescent reaction.

Results were reported according to the selected option, as described in the system operating instructions or in the online help system.

ETHICAL APPROVAL

The Animal ethics committee IAEC No: 001/NCP/IAEC/2015 approved the protocol, and appropriate measures were taken by our study group to minimize pain or distress in the animals [19,20]. The experimental segments of this study, the hormonal examination were performed at the local Laboratory as shown in Table 3.

Table 3: Hormonal examination.

Sl. No	Groups	LH mIU/mL	Testosterone ng/dL
1.	Normal	0.41 ± 0.09	1.203 ± 0.11
2.	Extract alone	0.56 ± 0.14	1.42 ± 0.35
3.	Control	0.16 ± 0.02	0.34 ± 0.09
4.	High dose ca	1.94 ± 0.22	3.22 ± 0.38
5.	Low dose ca	0.39 ± 0.17	0.673 ± 0.204

Serum testosterone results are as follows

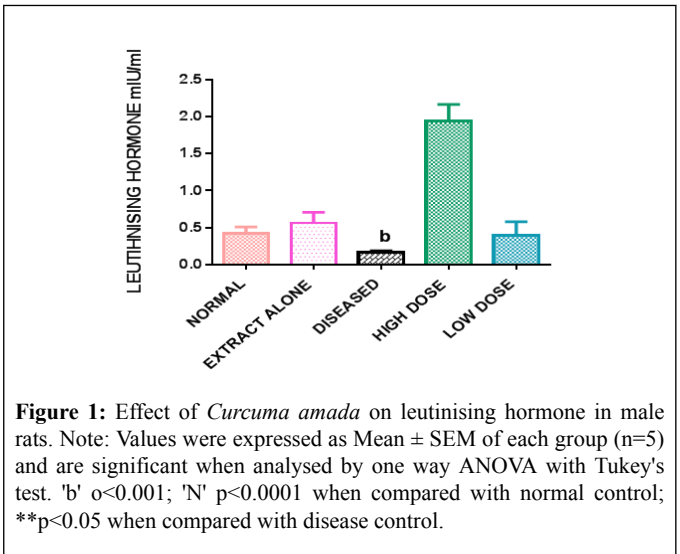

Figure 1: Effect of *Curcuma amada* on leutinising hormone in male rats. Note: Values were expressed as Mean ± SEM of each group (n=5) and are significant when analysed by one way ANOVA with Tukey's test. 'b' o<0.001; 'N' p<0.0001 when compared with normal control; **p<0.05 when compared with disease control.

LH assay results are as follows

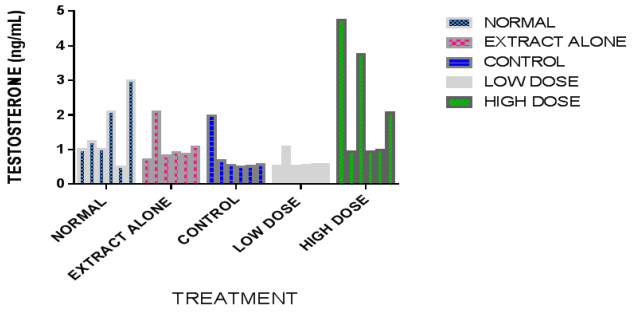

Figure 2: Effect of *Curcuma amada* on male rat hormone (Testosterone). Note: Values were expressed as Mean ± SEM of each group (n=6) and are significant when analysed by one way ANOVA with Geissear-Greenhouse's. 'control' p<0.1224 when compared with normal control; p<0.5396 when compared with disease control.

DISCUSSION

The present study was conducted to evaluate the protective effect of *Curcuma amada* on the male reproductive system of hormonal estimations like total testosterone and LH against mobile phone radiation-induced infertility in male Wister albino rats. Post-treatment with *Curcuma amada* 100 mg/kg, 200 mg/kg and 300 mg/kg body weight) significantly improved the levels of LH and testosterone hormonal levels in Wister rats.

In the Radiation alone groups was observed that the Hormonal levels were decreased, which was compared with Normal Group animals hormonal levels. The above discussion will be mainly on the radiation impact on Human beings that may leads to Hormonal imbalance.

CONCLUSION

Curcuma amada contains major chemical constituents: steroidal saponins, alkaloids and flavonoids. Steroidal saponins are Increase the levels of LH in testosterone, enhance the sexual behavior in male rats. Flavonoids are Increases in dehydroepiandrosterone. Alkaloids may Increase the release

of NO from endothelial and nerve endings of male rats, dilates the blood vessels in sexual organs of male rats Enhanced sexual performance.

Based on these earlier reports and the results of the present study; it was concluded that the presence of above compounds in *Curcuma amada* extract might be responsible for its protective role in radiation-induced infertility in male rats. In the present study, pretreatment with *Curcuma amada* (100 mg, 200 mg, and 300 mg/kg body weight) in that 100 mg/kg body weight without radiation extraction only. The hormone levels were also significantly increased.

Among the (200 mg/kg and 300 mg/kg) doses of *Curcuma amada*, the dose of 300 mg/kg of body weight showed significant protective effect than the dose 100 and 200 mg/kg body weight of *Curcuma amada* (mango ginger) extract. Furthermore, the protective effect of *Curcuma amada* was comparable with that of reference normal.

Calcium and phosphorus was proved to have a protective effect against mobile phone radiation-induced infertility in male rats by a significant increase in hormonal levels of serum testosterone and LH. In the Extract alone group statistical analysis was significantly increased in the LH and testosterone hormonal levels due to the absence of Mobile radiation and the only feed was *Curcuma amada* extract with 200 mg/kg.

From the result of the present study, it was concluded that the *Curcuma amada* has the protective effect on mobile radiation-induced infertility in male rats. The present study has shown that the effects of cell phone radiation and over-exposure to the human body can adversely affect cell health. This study is also proves that the radiation from Mobile Phones can affect the hormonal imbalance that leads to Infertility.

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