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PHARMACOLOGICAL INVESTIGATION OF SPERMATOGENIC POTENTIAL OF SIDDHA FORMULATION MANOKARA CHOORANAM AGAINST GENTAMICIN INDUCED TESTICULAR TOXICITY IN WISTAR RATS

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ABSTRACT

Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set-up like ours, with a strong emphasis on child-bearing. About 25 and 35 % of infertility is regarded to man and woman respectively. Male infertility accounts for about 50% of human infertility. In 40% to 50% of infertile males, the etiology is unknown. The pathophysiology of male infertility could be explained by a number of cellular abnormalities manifesting at the molecular and biochemical levels that result in decreased quality and quantity of sperm in the semen and an imbalance in the reproductive hormones. It has been widely documented that, in addition to conventional therapies, many individuals with sexual dysfunction often seek alternative therapies. Siddha system of medicine holds greater importance as it treats the infertility in human since ancient times. Manokara chooranam (MC) is a novel siddha formulation indicated for rejuvenation and reproductive cell proliferation as per the standard siddha literatures. It consists of several potential herbs which can acts by multiple mechanism. Main objective of the present investigation is to evaluate the spermatogenic activity of the drug MC in male wistar rats. Induction of oligospermia in male rats achieved by i.p injection of gentamycin 50mg/kg for the period of 30 days. Treatment with MC at the dose of 250 and 500 mg/kg exhibits higher proportion of sperm rejuvenation with increased sperm count followed by increased number of mature sperm with significantly improved viability and motility in experimental animals. In conclusion from the data's obtained from the present study the siddha drug MC has tendency to restore the spermatogenesis and may be considered as drug of choice in treating male infertility.

KEY WORDS: ***Male infertility, Oligospermia, Siddha system, Manokara chooranam, Spermatogenic activity, Sperm count***

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1. Introduction

Male infertility refers to a male's inability to result pregnancy in a fertile female. “Male factor” infertility is seen as an alteration in sperm concentration and/or motility and/or morphology in at least one sample of two sperm analyzes, collected 1 and 4 weeks apart [1]. In humans, it accounts for 40-50% of infertility [2-4] and affects approximately 7% of all men [5]. Male infertility is commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity [6]. According to the International Committee for Monitoring Assisted Reproductive Technology, World Health Organization (WHO), infertility is a disease of reproductive system defined by failure to achieve the clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [7]. Moreover, it has been widely observed that oligospermia is the single most prevalent cause of reduced male fertility [8,9].

Oligozoospermia, a medical condition characterized by low sperm count and quality is responsible for 90% of male infertility [10,11]. Notwithstanding, research findings have shown that not all men that demonstrate normal parameters in routine semen analysis are fertile. The hidden factor has been oxidative stress (OS) which is now recognized as an important and probable cause of idiopathic male infertility [12].

Broad range of strategies had been carried out to overcome the male infertility complications. Human chorionic gonadotropin (hCG) [13], treatment of follicle-stimulating hormone (FSH) [14], and usage of assisted reproduction technology (ART) are of the state-of-the-art options for these infertility issues [15]. However, shortcomings of the above stated treatments cannot be overlooked, which may include acceptability, high expenditure, and threat of diverse complications during surgical procedures. Approximately half of the couples undergoing consultation for male infertility terminated ART treatment, mainly due to the physical and emotional pain resulting from the treatment [16].

Siddha system of traditional medicine works behind the principle of rejuvenation with its phytocomponents as derived from several potential herbs. Bioactive phytotherapeutics offers tremendous influence on rapidly dividing sperm cells and aids in improving the sperm count, viability and motility. Manokara chooranam is a novel siddha formulation indicated for rejuvenation and reproductive cell proliferation as per the standard siddha literatures. It consist of several herbs such as *Vitis venifera, Hygrophila auriculata, Saraca asoca, Cycas circinalis, Cinnamomam veram, Syzygium aromaticum, Crocus sativus, Trigonella foenum, Trianthema decandra ,Mesua ferrea, Borneo camphor, Tribulus terrestris, Costus specisous, Mucuna pruriens ,Withaniya somminifera, Ziziphus mauritiania Cinnamomam tamala* and *Borassus flabellifer.* Still now there is no documentary research evidence on establishing this novel formulation for its sperm regenerative potential. Hence the main aim of the present study is to evaluate the sperm regenerative tendency of the siddha drug MC against gentamicin induced testicular toxicity in wistar albino rats.

2.Materials and Methods

2.1. Animals

Healthy adult Wistar albino male rats weighing between 220-240 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬+ 2o C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/X/090/2018

2.2. Animal grouping and Methodology

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Disease control group rats received Gentamycin 50mg/kg, i.p from day 1 to 30. Group III (low dose treatment group) animal received 250 mg/kg of MNC 1 hr prior to gentamycin injection from day 1 to 30. Group IV (High dose treatment group) animal received 500 mg/kg of MNC 1 hr prior to gentamycin injection from day 1 to 30.

2.3. Sample Collection

2.3.1. Determination of sperm count and motility [17]

24 hrs after last dose of drug administration the animals was sacrificed for the assessment of sperm count. The testis was being decapsulated by isolating cauda epididymis the sperm will be released by cutting into phosphate buffer saline and 0.5% bovine serum albumin of pH 7. The homogenate was under room temp for 5 min and an aliquot was taken in leukocyte hemocytometer and discharged in neuabaurs counting chamber and count will be done in 4 chambers. Remaining part of 0.3 ml of plain slide and the sperm

motility was analyzed with microscope. For morphological analysis PBS sperm suspension was admixed with 4 drops of eosin and thin smear of the same was made on to the glass slide and allowed to dry for further morphological analysis.

2.3.2. Determination of Sperm Morphology [18]

Homogenate of about 0.5 ml was stained with eosin at room temperature for 30 mins and the slide was observed under microscope for sperm morphology.

2.4. Histopathology Analysis [19]

Testes was dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation, a tissue was embedded in paraffin. Fixed tissues were cut at 10 µm and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs will be taken.

2.5. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3.Results

3.1. Effect of MC Sperm Count, Motility and Viability parameters on Gentamicin-Induced rats

Results analysis of sperm count, motility and viability of the rat sperm sample subjected to the examination has clearly revealed that there was a significant decrease in count and motility observed in gentamicin intoxicated rats when compare to that of the control group rats. Treatment with MC at the dose of 250 and 500 mg/kg revealed spermatogenic potential by significant increase in all the parameters such as count, motility and viability level. As shown in table 1 and figure 1 – 4.

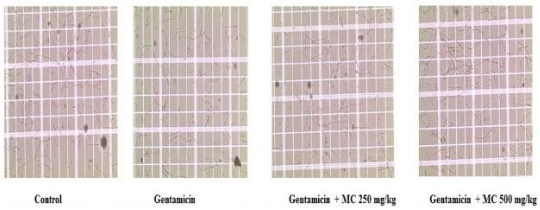

Figure 1: Microscopic observation of Sperm count using Neubauer's Chamber in control and treatment group rats

Table 1. Effect of MC Sperm Count, Motility and Viability parameters on Gentamicin-Induced rats

Group	Sperm Count X 10 ^{6}	Sperm Motility (%)	Viability (%)
Control	73.33 ± 1.02	87.83± 2.54	78.17 ± 2.84
Gentamicin intoxicated rats	35.33 ± 2.02*	26.17± 2.43**	20.33 ± 2.29**
Gentamicin + MC 250 mg/kg,(p.o)	46.83 ± 1.55	48.42 ± 1.95*	43.0 ± 2.54*
Gentamicin + MC 500 mg/kg,(p.o)	53.83 ± 1.16*	59 ± 2.19*	66.83 ± 2.67*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

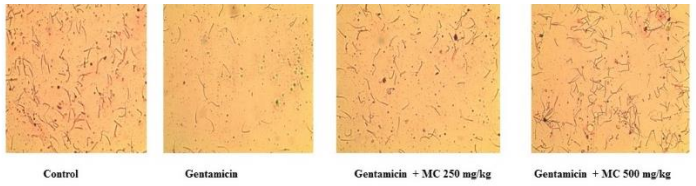

Figure 2: Microscopic observation of Sperm morphology in control and treatment group rats under low magnification

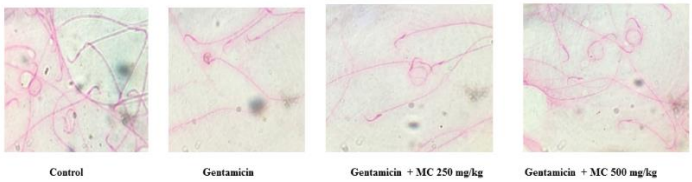

Figure 3: Microscopic observation of Sperm morphology in control and treatment group rats under high magnification

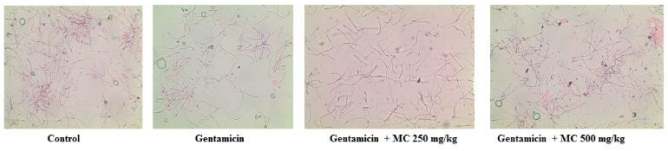

Figure 4: Microscopic observation of Sperm motility in control and treatment group rats under high magnification

3.2. Effect of MC on histopathology of rat testes in control and treatment group

Light microscopic observation reveals the presence of dense volumes of normal sertoli cell properly aligned on the basement membrane with oval dome shaped nucleus were observed in group I. Further regular histology of seminiferous tubule was observed in this group. There was significantly reduction in the density

of primary spermatocytes with distorted orientation and evidence of interstitial fibrosis was observed in group II samples.Marginal increase in the number of spermatids on the lumen of seminiferous tubules with regular arrangement of sertoli cells were observed in sample belongs to group III. Arrangement of Interstitial tissues with restored morphology of sertoli cells were observed, increased density in the number of primary spermatocytes with large centered nucleus and dense chromatin were observed in sample belongs to group IV.

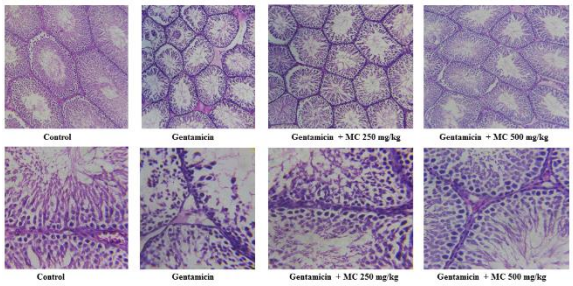

Figure 5: Effect of MC on Histopathology of Testes in control and treatment group rats under low and high power magnification

4.Discussion

The spermatozoa are susceptible to oxidative stress induced damage because of the large polyunsaturated fat content in their membranes [20]. In recent years, many studies revealed that gentamicin induces an oxidative stress-status in the testis by increasing free radical formation and lipid peroxidation. These biochemical changes manifest as structural and cytotoxic changes in the testis. Further, gemtamicin affects the spermatozoa by affecting their number, motility and morphology [21,22]. The sperm count and sperm motility were decreased and abnormality was increased.

Gentamicin induced structural changes such as sloughing of somniferous epithelium, vacuoles and gaps in the epithelium, nuclear pyknosis and atrophic changes in a few tubules [23,24]. Antibiotics like gentamicin routinely used by urologists, andrologist and to treat infections prior to in vitro fertilization treatment or when high concentration of leukocytes is present in the semen of these patients [25,26].

It was observed from the present investigation that there was a significant decrease in the sperm count of rats injected with gentamicin when compare to the control group which denotes the induction of testicular toxicity in the experimental animals, Treatment with MNC at both the dose level exhibits higher proportion of sperm rejuvenation with increased sperm count in rats belongs to group III and IV. Morphology of sperm appears normal with regular arrangement of acrosome to tail structure observe in group I rats. Higher proportion of pin headed sperm with immature spermatids were observed in gentamycin alone treated group II. Uniformity in sperm chord with prominent angle on head were observed in sperm sample belongs to group III and IV

Increased percentage of non-motile sperm with sluggish motility and reduced percentage of viability were observed in rats belongs to group II. Marginal increase in percentage of mature sperm with significantly improved viability and motility were observed in rats treated with MNC at both the dosage level.

Present treatment for male infertility includes the usage of selective estrogen receptor modulator (SERM) that is found in a racemic mixture of two isoforms—enclomiphene and zuclomiphene [27]. This class of medications competitively binds to the estrogen receptors on the hypothalamus and pituitary gland thereby negating the negative feedback imposed by estrogen. Luteinizing hormone (LH) secretion is subsequently increased resulting in more testosterone production by the testes [28].

The effects of aromatase inhibitors on spermatogenesis have also been investigated. In a single case report of nonobstructive idiopathic azoospermia, daily 2.5 mg letrozole for 4 months was able to convert a patient with hypospermatogenesis to active spermatogenesis proven by testicular biopsy [29]. The use of Human chorionic gonadotropin (HCG) for the treatment of male infertility is most commonly applied to the treatment of patients presenting with hypogonadotropic hypogonadism (HH). The mechanism of action of HCG involves its action as an analogue of LH and its subsequent role in maintaining and/or increasing intratesticular levels of testosterone [30].

There are several medicinal plants that could be used in combination with assisted reproductive technology (ART) to lower the cost and increase the success rate of infertility treatment [31]. Using herbs in this way is not a new idea. As infertility treatment is expensive

and has a low success rate – approaching 49% with cumulative attempts [32].

Histopathological observation of control rats reveals dense volumes of normal sertoli cell properly aligned on the basement membrane with oval dome shaped nucleus were observed in group I. Further regular histology of seminiferous tubule was observed in this group. There was significantly reduction in the density of primary spermatocytes with distorted orientation and evidence of interstitial fibrosis was observed in group II samples. Treatment with siddha formulation MC shown marginal increase in the number of spermatids on the lumen of seminiferous tubules with regular arrangement of sertoli cells were observed in sample belongs to group III. Arrangement of Interstitial tissues with restored morphology of sertoli cells were observed, increased density in the number of primary spermatocytes with large centered nucleus and dense chromatin were observed in sample belongs to group IV.

5.Conclusion

Male infertility is considered to be one of the most important crisis among the younger generation of reproductive age group. The stigma, costs, and uncertainty associated with biomedical fertility treatments often entice those who are having problems conceiving to use alternate medicine as a first line of treatment before engaging in more medically invasive treatment procedures. Treatment with MC at the dose of 250 and 500 mg/kg exhibits higher proportion of sperm rejuvenation with increased sperm count followed by increased number of mature sperm with significantly improved viability and motility in experimental animals. In conclusion from the data's obtained from the present study the siddha drug MC has tendency to restore the spermatogenesis and may be considered as drug of choice in treating male infertility.

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