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Original Article

Anti-infertility activity of N-Miracle (poly herbal formulation) in ethanol induced infertility in male albino rats.

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ABSTRACT

Aim: This study was performed for determining the ability of N-Miracle (poly herbal formulation) to increase aphrodisiac activity and to cure ethanol induced germ cell damage and infertility in male rat models. **Methods:** Different concentration of the drug against aqueous ethanol induced testicular damage was done on male albino rats. Hematological parameters, sperm count, serum testosterone, Estrogen, Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH) were investigated. **Results:** Dosage of 20mg/kg of N-Miracle showed a significant reversal ($P < 0.05$) in hematological parameters. The fertility factors such as total body weight, sperm motility and hormonal changes were back to normal levels. Significant increases in sperm count were observed in drug treated group as compared with the ethanol treated infertility group. Similarly, reductions in the percentage of abnormal sperm were noted in animals treated with drug. **Conclusion:** N-Miracle showed aphrodisiac activity on induced infertility in male rats.

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1. Introduction

Various factors contribute for sexual dysfunction like psychological disorders, lifestyle, systemic diseases etc., (1) An aphrodisiac is defined as an agent (food or drug) that arouses sexual desire. Several types of treatment are claimed in the modern medicine, but due to being expensive and adverse effects, search of natural supplement as an aphrodisiac substance is significantly increased. The possibility of bioactive aphrodisiacs, which may be derived from plants, animals or minerals, has been attractive throughout recorded history. Stating this, medicinal plants and herbal medicines has become part and parcel of human society to combat both infectious and non-infectious diseases. Plant-derived chemicals are used to relieve sexual dysfunction and they have sex enhancing potentials. These phytochemicals increase libido, sexual potency and sexual pleasure (2, 3). Therefore, the present study was undertaken to determine the aphrodisiac activity of N-Miracle, which is a newly formulated herbal mix on the sexual behavior in male rats.

2. Material and Methods

2.1. Animals:

Male Wistar albino rats weighing 150-200 grams were used for the study. The animals were fed with commercial pellet feed, under the brand name "Gold Mohur Feed" and water was given ad libitum. The animals were subjected to a 12:12 h light: dark cycle under standard laboratory conditions at a temperature of 24-28 °C

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with a relative humidity of 60%-70%. The experiments were conducted as per the guidelines of the Institutional Animal Ethical Committee, New Delhi (CPS/IAEC/AH/P/19/20).

New heading: Combination of N-Miracle (Poly herbal formulation):

1. Conium maculatum
2. Lycopodium clavatum.
3. Selenium.
4. Vitex agnus-castus.
5. Yohimbinum.
6. Caladium Seguinum

2.2. Experimental groups

The male rats were divided into 4 groups, each consisting of 6 rats. All the groups except group I and group II was fed with 20% v/v ethanol for 30 days to induce testicular injury. Group I: This group served as the control. Group II: Drug control- Animals received a single daily dose of 20 mg/kg for 30 days. Group III: This group served as the negative control and received ethanol (20%v/v, 1.6 g/ kg body weight/day) for 30 days to induce testicular damage. (4) Group IV: Treatment group- Animals after induced testicular damage, received drug 20 mg/kg orally, as a single dose daily for 30 days.

2.3. Recording of the Body Weights

To determine the effect of extract on the body weight, all the adult male rats were weighted before the start and at the end of the dosing schedule. Weights were recorded with the small animal weighing machine.

2.4. Determination of Organ Weight

During the sacrificing of the adult male rats, the Testis, Seminal vesicle and Epididymis were removed, blotted on a filter paper and weighed accurately.

2.5. Determination of Sperm Count

The samples for these studies were obtained by making small cuts in the cauda epididymis and vas deferens. The original suspension of sperms was thoroughly shaken to evenly disperse the sperms. 0.05 ml of the sample was diluted to 1 ml (1:20 Dilution) with formalin bicarbonate solution using, white blood cell pipette. The formalin bicarbonate solution effectively immobilizes the sperm cell to facilitate counting of the sperms with accuracy. Counting was done on a haemocytometer using the usual RBS counting chamber (5).

2.6. Evaluation of Sperm Motility

Suspension of the spermatozoa was shaken and diluted with Phosphate Buffer Saline (pH 7.4 Temperature 37°C). To evaluate sperm motility, a small drop of spermatozoa suspension was placed on a 37°C pre-warmed microscope slide approximately and then covered with a cover slip ringed with petrolatum. The microscope slide was observed under high power. Motility was evaluated by scanning 10 different fields containing at least 10 sperms. Accordingly the sperm sample was rated as having high motility, moderate motility, low motility or non motile (5).

2.7. Haematological parameters

Hematological parameters RBCs, hemoglobin, WBC and platelets were analyzed by standard techniques described by Baker et al., 1998 (6).

2.8. Hormonal assay

The serum testosterone, estrogen, LH and FSH was quantitatively assayed by direct human serum testosterone enzyme immunoassay kit according to manufacture's procedure.

2.9. Statistical analysis

Data are expressed as mean \pm S.D. and analyzed for statistical significance by using one way analysis of variance (ANOVA). Results were considered significant at the $P \leq 0.05$ level.

3.0. Results

Table 1. Effect of N-Miracle on the Whole body weight in different experimental groups

Group	Body Wt in grams(g)	
	I day	After Inducing & treatment
Control	155 \pm 3.5	182 \pm 6.6a
Drug control (20mg/kg bw)	158 \pm 4.4	186 \pm 7.8a
Infertility	156 \pm 5.8	121 \pm 4.5b
Infertility + Drug (20mg/kg bw)	153 \pm 3.7	188 \pm 7.8a

Values are means of \pm SD of 6 rats

Values not sharing a common superscript differ significantly at $P < 0.05$ DMRT

Table 2 Effect of N-Miracle on the Organ weight in different experimental groups

Group	Testis (mg)	Epididymis(mg)	Seminal vesicle(mg)
Control	1100 \pm 23.5a	398 \pm 8.23a	360.5 \pm 9.21a
Drug control (20mg/kg bw)	1065 \pm 20.5a	406 \pm 8.0a	368 \pm 8.0a
Infertility	730 \pm 12.8b	270 \pm 6.1b	238.67 \pm 6.6b
Infertility + Drug (20mg/kg bw)	1038 \pm 18.0a	380 \pm 9.2a	351.26 \pm 7.8a

Values are means of \pm SD of 6 rats

Values not sharing a common superscript differ significantly at $P < 0.05$ DMRT

Table 3 Effect of N-Miracle on percentage motility and sperm count in different experimental groups

Group	Testis millions/mm3	Cauda Epididymis millions/mm3	Sperm motility%- Cauda Epididymis
Control	4.8 \pm 0.30a	61.12 \pm 2.8a	55.5 \pm 3.0 a
Drug control (20mg/kg bw)	5.0 \pm 0.23a	60.7 \pm 2.33a	57.8 \pm 2.8a
Infertility	2.8 \pm 0.11b	34.4 \pm 1.8b	33.25 \pm 4.1b
Infertility + Drug (20mg/kg bw)	4.7 \pm 0.15a	59.8 \pm 3.3a	56.8 \pm 3.45a

Values are means of \pm SD of 6 rats

Values not sharing a common superscript differ significantly at $P < 0.05$ DMRT

Table 4 Effect of N-Miracle on Hematological parameters in different experimental groups

Groups	RBC millions/mL	WBC thousands/mL	Platelets Lakhs/mL	Hb (g/dL)
I	5.85 \pm 0.24a	7.67 \pm 0.32a	4.80 \pm 0.15a	16.35 \pm 1.50a
II	5.54 \pm 0.28a	7.51 \pm 0.33a	4.94 \pm 0.25a	15.82 \pm 1.19a
III	3.70 \pm 0.22b	5.89 \pm 0.40b	2.98 \pm 0.23	12.90 \pm 2.54b
IV	5.80 \pm 0.24a	7.58 \pm 0.28a	4.70 \pm 0.20a	16.10 \pm 0.55a

Values are means of \pm SD of 6 rats

Values not sharing a common superscript differ significantly at $P < 0.05$ DMRT

Table 5 Effect of N-Miracle on Serum Testosterone, Estrogen, LH, and FSH in different experimental groups

Group	Testosterone ng/mL	Estrogen pg/mL	LH μ IU/mL	FSH μ IU/mL
I	5.11 \pm 0.20	24.70 \pm 2.0	4.10 \pm 0.15	1.22 \pm 0.07
II	5.10 \pm 0.18	25.10 \pm 3.2	3.97 \pm 0.16	1.28 \pm 0.08
III	3.25 \pm 0.10	26.10 \pm 1.80	6.70 \pm 0.10	2.20 \pm 0.05
IV	5.05 \pm 0.21	24.80 \pm 2.80	4.07 \pm 0.21	1.24 \pm 0.07

Values are means of \pm SD of 6 rats

Values not sharing a common superscript differ significantly at $P < 0.05$ DMRT

The aqueous solution of N-Miracle at concentration of 20mg/Kg body weight was treated on male Wistar albino rats for ethanol induced infertility.

Except infertility control other groups showed no significant change in body weight. The total body weight was significantly decreased ($P < 0.05$) in Group III animals in comparison with other groups. Group III, which received ethanolic exposure for 30 days for testicular damage. Ethanol exposed rats (Group III) alone showed significant decrease in all accessory sex organs namely testis, epididymis and seminal vesicle.

Hematological parameter, RBC and WBC counts, hemoglobin, and platelets varied within the normal range in control, infertility treated and drug control groups. Where as infertility control group exhibited variations.

Sperm count and sperm motility of caudal epididymis and testis, in infertility treated group was within the normal range. While there was a sharp decrease in infertility control group. Reproductive hormones of our interest testosterone, LH, estrogen and FSH showed a positive response in drug treated infertility rats.

4. Discussion

Administration of the drug resulted in weight gain in treated animals. Testes and epididymal weights were also increased significantly. The increase in body and organ weights was comparable with testosterone. Since androgenic effect is attributable to testosterone levels in the blood, it is likely that the drug may have a role in testosterone secretion allowing better availability of hormone to gonads (7). A significant anabolic effect upon administration of the drug N-Miracle was observable as compared to the control group, which was comparable to that of the administration of testosterone suggesting a testosterone type action of the drug. This investigation clearly suggests anabolic steroidal effect of N-Miracle.

The development of normal and mature sperm is the key to optimum male fertility. The production of sperms and testosterone in the testes are mainly regulated by FSH and LH, which are released from anterior pituitary (8,9).

FSH stimulates spermatogenesis and LH stimulates synthesis and release of testosterone. Testosterone causes an increased blood flow and stimulates the growth of the target tissues. Testosterone cause direct stimulation of spermatogenesis. Our results also show that there is increase in spermatogenesis and increase in weight of sexual organ in drug treated group as comparison to infertility induced control group (10). The decrease in sperm count and percentage of motility in infertility animals is due to free radical injury to the spermatozoa (11,12).

It is well established that hematological testes form the very front-line investigation on which diagnosis of various diseases is based. A significant increase in the final body weight and unaltered hematological parameter in any of the treatment group in the present exploration in rats, suggest that the N-Miracle does not cause any adverse effect on general health of the animals.

5. Conclusion

Sexual behavior was studied in normal, infertility rats to understand the role of N-Miracle as an aphrodisiac. There was an overall positive response in the physiology of sexual behavior. These results were statistically significant. It is concluded that N-Miracle appears to possess aphrodisiac activity.

References

1. Guay AT, Spark RF, Bansal S, Cunningham GR, Goodman NF, Nankin HR et al. American association of clinical endocrinologist, Medical guidelines for clinical practice for the evaluation and treatment of male sexual dysfunction, A couple's problem. *Endocrinol pract* 2003; 9(1):78- 95.
2. A.F. G. Cicero, E. Bandieri and R. Arletti. "Lepidium meyenii Improves Sexual Behaviour in Male Rats Independently from Its Action on Spontaneous Locomotor Activity," *Journal of Ethnopharmacology* 2001;75(2-3): 225-229.
3. A.Adimoelja, "Phytochemicals and the Breakthrough of Traditional Herbs in the Management of Sexual Dysfunctions," *International Journal of Andrology* 2000; 23(2):82-84.

4. Maneesh M, Jayalakshmi H, Sanjiba D et al. Experimental therapeutic intervention with ascorbic acid in ethanol induced testicular injuries in rats. *Indian Journal of Experimental Biology* 2005;43: 172-176
5. Zaneveld LJD and Pelakoski: Collection and physical examination of ejaculate. In:Hafez (ESE) Ed.Techniques in human andrology. Vol I Human Reproductive medicine. North Holland Publishing company, Amsterdam,1997;147-172.
6. Baker et al., Baker and Silverton's Introduction to Medical Lab Technologies seventh edition 1998;356-360.
7. Thakur M, Dixit VK. Effect of Chlorophytum borivilianum on androgenic & sexual behavior of male rats. *Indian Drugs* 2006; 43: 300-306.
8. Steinberger E. Hormonal control of mammalian spermatogenesis. *Physiological Review* 1971; 1-22:5111
9. Zarrow MX, Yochim JM, McCarthy JL. *Experimenatal endrocrinology, A sourcebook of basic technique.* Academic Press, New York and London. 1964.
10. Anderson Jr RA, Willis BR, Oswald C et al. Ethanol induced male infertility: Impairment of Spermatozoa. *Journal of Pharmacology and Experimental Therapeutics* 1983;225: 479-485
11. Madhusudana Reddy C, Ramakrishna CM, Saraswati BP. Antispermatoxic and Androgenic activities of various extract of Hibiscus rosasinensis in albino mice. *Indian Journal of Experimental Biology* 1997;35: 1170-1174
12. Yakubu MT, Akanji MA, Oladiji A. Male sexual dysfunction and methods used in assessing medicinal plants with aphrodisiac potentials. *Phcog Rev* 2007; 11: 49-56.