

ASSESSMENT OF ACUTE TOXICITY AND REPRODUCTIVE CAPABILITY OF A HERBAL COMBINATION

AZRA RIAZ, RAFEEQ ALAM KHAN*, SHADAB AHMED AND SYEDA AFROZ

Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan

ABSTRACT

The drug under investigation is an herbal combination of *Withania somnifera*, *Tribulus terrestris*, *Mucuna Pruriens* and *Argyreia speciosa* which has been used for several years of its bio-stimulating, revitalizing and fertility enhancing effects. Present preclinical study is specifically designed to access the safety and efficacy of the product. The result of acute oral toxicity reveals that product is safe up to the dose of 5000mg/kg. The effects of study related to reproductive capability of drug on both sex reveals increase in reproduction rate up to two generations i.e. F₀ and F₁.

Keywords: *Withania somnifera*, *Tribulus terrestris*, *Mucuna pruriens*, *Argyreia speciosa*, acute oral toxicity, reproductive capability.

INTRODUCTION

Herbal medicines have been extensively used in developed countries hence they are natural and relatively safe (Gurib-Fakim, 2006). They contain plant materials as their pharmacologically active components (Pribitkin, 2005). Plants and derivatives of plant played a key role in world health and have long been known to possess biological activity. Thirty percent of all modern drugs are derived from plants (Burns, 2000). According to the World Health Organization about 80% of the world's population living in developing countries relies essentially on plants for primary health care (McKay *et al.*, 2007). Herbal medicine associated pharmacology and pharmaceutical products are updated frequently (Wang *et al.*, 2002). At present time, it is easier to determine efficacy and safety of herbal remedies, because it is known which chemical compounds are present in these plants and which of these compounds are associated with a number of side effects (Rodriguez-Fragoso *et al.*, 2008). The drug under study i.e., is a combination of medicinal plants including, *Tribulus terrestris*, *Mucuna Pruriens*, *Argyreia speciosa* and *Withania somnifera* or *Ashwagandha*.

Infertility is a complex disorder with significant medical, psychosocial and economic aspects. Medications can be administered to induce follicular development and ovulation (Chandra *et al.*, 1998). Ovulatory and immunologic are some of factors that contribute to infertility. Spermatozoa are relatively fragile cells and are easily damaged by a number of environmental and life style habits (Glatstein *et al.*, 1998).

A variety of products and treatment including vitamins and Chinese herbs have been used but there are very few scientific data available to support the benefits of these

agents in infertility therapy (Teng *et al.*, 2008). Thus complete evaluation of the effectiveness and efficacy of herbal medicines might require randomized clinical trials.

Present preclinical study is designed to access the safety and efficacy of the herbal product. This reproduction study evaluates its effects on reproductive systems and capability of Albino mice. Their postnatal maturation, reproductive capacity of offspring and possible cumulative effects through several generations were also observed. It also provides information concerning the effects of herbal drug on mating behavior, gestation, neonatal morbidity, mortality, lactation, weaning, growth and development of the offspring (FDA, 2000).

MATERIALS AND METHODS

Dosing

500 mg capsule contain *Tribulus terrestris* 300 mg, *Withania somnifera* 100 mg, *Mucuna pruriens* 25 mg, *Argyreia speciosa* 60 mg & 15 mg Talc as excipient was dissolved in 10% DMSO and administered in the dose of 27 mg/kg through oral route. Control group received 10% DMSO orally equivalent to the volume of respective doses according to their body weight.

Acute oral toxicity LD₅₀

Mice of both sexes with uniform weight have been used for the determination of LD₅₀. Females used were nulliparous and non-pregnant. At the commencement of dosing, age of each animal ranged between 6 to 8 weeks. The drug was administered I/P to healthy fasted rodents through single bolus dose of herbal extract using 10% DMSO as vehicle. Animals were arranged in three groups, each consist of 3 animals. Each group was Administered 10,100 and 1000mg/kg of herbal extract. Toxic signs and the severity, onset, progression and reversibility of the

Corresponding author: e-mails: rkhan1959@gmail.com, rafkhan@uok.edu.pk

signs have been observed and recorded in relation to dose and time. The animals were observed continuously for 24 to 48 hr after dosing. The next dose was scheduled according to Lorke's method on the basis of outcomes i.e., number of animals died and then acute oral toxicity was calculated (Lorke, 1983).

Fertility evaluation

This study was administered in a normal dose of 27mg/kg/day to parental (F_0 generation) male and female mice, prior to and during mating, gestation and through the weaning of F_1 offspring. The same concentration of drug was then given to selected F_1 generation offspring during their growth and development to adulthood and through the mating period. Pregnant F_1 generation females continue to receive the test substance throughout gestation, until the F_2 generation offspring were weaned (FDA, 2000).

Five pairs of healthy albino mice having uniform weight and age were used for both generations. The females were not pregnant and nulliparous. Animals were assigned to control and treated groups in a random manner to minimize bias. All treated and control animals were acclimated to the study conditions five days before treatment begins. The volume of vehicle given to control animals was equal to the maximal amount of vehicle given to dosed group. Animals were exposed to the test substance through oral route during the entire study period.

For each mating, a female was placed with a single randomly selected male till the pregnancy occurs and throughout their whole gestational period. Near parturition, pregnant females were caged separately. One male and one female were randomly selected from each litter for mating with another pup of the same group to produce the next generation. The mating procedures for the F_1 males and females were carried out in the same manner as the F_0 parental animals. Each litter was examined as soon as possible after delivery for the number of pups, stillbirths, live births, and the presence of gross anomalies. The neonates were carefully observed on postnatal days zero (day of birth), four, seven, fourteen, and 21. Dead pups were macroscopically examined for possible gross defects and the cause of death. End points of reproductive toxicity expressed the animal's responses to the study drug from conception to weaning.

Data on the average number of pups that survived during a specific interval (e.g., average number of pups that survived from birth to day four, or the average number of pups that were weaned) was examined. This analysis considers the total effect of the test substance at all stages to that point which is a more sensitive indicator for reproduction studies.

RESULTS

Acute oral toxicity LD_{50}

Herbal combination exhibits LD_{50} values greater than 5gm/kg per 24 hr. All animals tolerated doses of herbal combination up to 5gm/kg during 24 hr period.

Fertility evaluation

Table 1 and 2 reveals the pharmacological evaluation of F_0 and F_1 generation of albino mice orally administered with normal dose of herbal combination against control group.

Table 1: Multigenerational reproduction analysis of herbal combination treated and control animals F_0 generation

Parameters	F_0 Treated	F_0 Control	% Increase F_0 Treated
No. of pregnancies	11	10	10%
No. of pups born	68	64	06%
No. of pups alive day 0	63	56	05%
No. of pups alive day 21	46	31	19%
Total male pups	34	32	--
Total female pups	34	32	--

Table 2: Multigenerational reproduction analysis of herbal combination treated and control animals F_1 generation

Parameters	F_1 Treated	F_1 Control	% Increase F_1 Treated
No. of pregnancies	09	08	11%
No. of pups born	58	47	19%
No. of pups alive day 0	52	41	02%
No. of pups alive day 21	36	23	13%
Total male pups	29	23	--
Total female pups	29	24	--

Animals of F_0 generation treated with study drug showed approx 10% increase in total pregnancies. There was nearly 10% increase in total no of pregnancies, 6% increase in total no. of pups born, 5% increase in early survival (pups alive on day 4) and 19% increase in over all weaning and growth (pups alive day 21) in comparison with control group animals. No significant change has been observed in conception, gestation period, sex ratio and average number of pups born in each successful pregnancy.

Where as in F_1 generation, approx 11% increase have been observed in total no of pregnancies, 19% increase have been observed in total no of pups born, 2% increase in early survival and the over all weaning and growth of F_1 offspring treated with study drug was observed to be increased by 13 % against F_1 control group. All related

parameters like conception, gestation period, sex ratio and average number of pups born per pregnancy were almost similar in comparison with the F₁ control group.

DISCUSSION

Medicinal plants behave as authentic medicines because the chemical substances of which they are formed can have a biological activity in humans. Determination of efficacy and safety of herbal remedies is necessary because many people using these agents as self-medication (Rodriguez-Fragoso *et al.*, 2008). Although there is a limited data available about the pharmacology and toxicology for the most commonly used herbal remedies (Gurib-Fakim, 2006). Therefore, efforts to elucidate health benefits and risks of herbal medicines should be intensified. Current study was design to assess safety and efficacy of herbal medicine. The result of acute toxicity after oral administration reveals the LD₅₀ value greater than 5000mg/kg. These findings suggest that an herbal combination is comparatively safe and does not possess acute untoward effects.

The drug under investigation herbal combination has been used for several years of its bio-stimulating, revitalizing and fertility enhancing effects (Gauthaman *et al.*, 2002). Reproductive capability of this herbal product has been revealed an overall improvement in fertility, reproductive health and development of animals.

Enhanced fertility and overall improvement in survival of pups may be due to the presence of *Tribulus terrestris*, *Withania somnifera* and *Mucuna Pruriens* in the formulation.

Several studies shows that *Tribulus terrestris* has the ability to improve some aspects of male sexual behavior by increasing testosterone levels and muscle strength by raising blood levels of another hormone i.e luteinizing hormone and enhance spermatogenesis in rats (Gauthaman *et al.*, 2002, 2003; Park *et al.*, 2006). So it could be recommended in cases of spermatorrhea (Rowland and Tai, 2003). It also has stimulatory effects on sperm quantity and quality and improved sexual response in men (Arsyad, 1996). Increased androgen levels to rats and rabbits (Gauthaman *et al.*, 2008) and recent report of positive effect on rat sperm production with unchanged levels of circulating androgens to rats have also been reported (Martino-Andrade *et al.*, 2009).

Similarly Shappira (1987) and Bhattacharya (1995) reported improve reproductive capability in both sexes using animal model by *Withania somnifera* (*Ashwagandha*). Several studies on this plant indicated that it possesses anti-inflammatory, anti-stress, antioxidant properties (Mohanty *et al.*, 2008) besides positively influencing the endocrine system (Mishra *et al.*, 2000; Visavadiya *et al.*, 2007). Male infertility associated with

the presence of oxidative stress, excessive production of reactive oxygen species and decrease in antioxidant defenses. Lifestyle factors and immunologic factors are other causes of male infertility (Horwards, 1995). Since fertility enhancement effect of herbal combination might be due to presence of *Ashwagandha* because it is considered to be an adaptogen, facilitating the ability to withstand stressors (Archana *et al.*, 1999) and has antioxidant properties as well (Bhattacharya *et al.*, 2001; Jaleel *et al.*, 2008).

Mucuna Pruriens also has the ability to regulate the steridogenesis and improves semen quality (Mahdi *et al.*, 2008).

CONCLUSION

Data collected during this study is insufficient to reach at a definite conclusion since trial in humans is required to establish safety and efficacy of any drug. However this study justifies the traditional use of herbal combination in sexual dysfunction and other fertility disorders, though further research is necessary to better understand the role of herbal combination to improve fertility and prevent infertility disease.

ACKNOWLEDGEMENT

Authors are thankful to Herbion Pharmaceuticals for providing the financial assistance and herbal combination for the study.

REFERENCES

- Akerele O (1993). Summary of WHO guidelines for the assessment of herbal medicines. *Herbal Gram*, **28**: 13-19.
- Aphale AA, Chhibba AD and Kumbhaakarna NR (1998). Subacute toxicity study of the combination of ginseng (*Panax ginseng*) and ashwagandha (*Withania somnifera*) in rats: a safety assessment. *Indian J. Physiol. Pharmacol.*, **42**: 299-302.
- Archana R and Namasivayam A (1999). Antistressor effect of *Withania somnifera*. *J. Ethnopharmacol.*, **64**: 91-93.
- Arsyad KM (1996). Effect of protodioscin on the quantity and quality of sperms from males with moderate idiopathic oligozoospermia. *Medika*, **22**: 614-618.
- Aslani MR, Movassaghi AR, Mohri M, Pedram M and Abavisani A (2003). Experimental *Tribulus terrestris* Poisoning in sheep: Clinical, laboratory and pathological finding. *Vet. Res. Commun.*, **1**: 53-62.
- Bhattacharya A, Ghosal S and Bhattacharya SK (2001). Antioxidant effect of *Withania somnifera* glyco-withanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol.*, **74**: 1-6.

- Bhattacharya SK (1995). Compromised reproductive function-restoring eEffect. *Phytotherapy Research*, **9**: 110-113.
- Burns MM (2000). Alternative medicine: Herbal preparation. *Clin. Ped. Emerg. Med.*, **1**: 186-190.
- Chandra A and Stephen EH (1998). Impaired fecundity in the United States: 1982-1995. *Fam. Plann. Perspect.*, **30**: 34-42.
- Dhan P, Abhishek N and Tewari SK (2001). Nutritional properties of *Mucuna pruriens*. *International Journal of Food, Science and Nutrition*, **52**: 79-82.
- Eisenberg DM, Davis RB, Ettner SL, Susan LE, Scott A, Wilkey S, Rompay MV and Kersler RC (1998). Trends in alternative medicine use in the United States, 1990-1997: results of a follow-up national survey. *JAMA*, **280**: 1569-1575.
- FDA, Guidelines for Reproduction Studies FDA/CFSAN Redbook 2000 IV.C.9.a.
- Gagnier JJ, DeMelo J, Boon H, Rochon P and Bombardier C (2006). Quality of reporting of randomized controlled trials of herbal medicine interventions. *Am. J. Med.*, **119**: 1-11.
- Gauthaman K and Ganesan AP (2008). The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction – an evaluation using primates, rabbit and rat. *Phytomedicine*, **15**: 44-54.
- Gauthaman K, Adaikan PG and Prasad RN (2002). Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats. *Life Sciences*, **71**: 1385-1396.
- Gauthaman K, Adaikan PG and Prasad RN (2002). Aphrodisiac properties of *Tribulus terrestris* in normal and castrated rats. *Life Science*, **71**: 1385-1396.
- Gauthaman K, Ganesan AP and Prasad RN (2003). Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): An evaluation using a rat model. *Journal of Alternative and Complementary Medicine*, **9**: 257-265.
- Glatstein IZ, Harlow BL and Hornstein MD (1998). Practice patterns among reproductive endocrinologists: further aspects of the infertility evaluation. *Fertil Steril*, **70**: 263-269.
- Gokhale AB, Damre AS, Kulkarni KR and Saraf MN (2002). Preliminary evaluation of anti-inflammatory and anti-arthritis activity of *S. lappa*, *A. speciosa* and *A. aspera*. *Phytomedicine*, **9**: 433-437.
- Gurib-Fakim A (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol. Aspects Med*, **27**: 1-93.
- Horwards SS (1995). Current concepts: Treatment of male infertility. *New England J. Med.*, **332**: 312-317.
- Jaleel CA, Gopi R, Manivannan P and Panneerselvam R (2008). Exogenous application of triadimefon affects the antioxidant defense system of *Withania somnifera* Dunal. *Pesticide Biochemistry and Physiology*, **91**: 170-174.
- Lorke D (1983). A new approach to practical acute toxicity testing. *Archive of Toxicology*, **54**: 275-287.
- Mahdi AA, Shankwar SN and Ahmad MK (2008). Effect of *Mucuna pruriens* on hormonal status and Semen quality in infertile males. *Contraception*, **78**: 167-195.
- Martino-Andrade AJ, Morais RN, Spercoski KM, Rossi SC, Vecchi MF, Golin M, Lombardi NF, Greca CS, Dalsenter PR (2009). Effects of *Tribulus terrestris* on endocrine sensitive organs in male and female Wistar rats. *Journal of Ethnopharmacology*, (In Press).
- McKay DL and Blumberg JB (2007). A review of the bioactivity of South African herbal teas: Roobos (*Aspalathus linearis*) and Honey comb (*Cyclopia intermedia*). *Phytother. Res.*, **21**: 1-16.
- Mishra L, Singh BB, Dagenais S (2000). Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. *Alter. Med.*, **5**: 334-346.
- Mohanty IR, Dharamvir SA and Gupta SK (2008). *Withania somnifera* provides cardioprotection and attenuates ischemia/reperfusion induced apoptosis. *Clinical Nutrition*, **27**: 635-642.
- Park SW, Lee CH and Shin DH (2006). Effects of SA1, a herbal formulation, on sexual behavior and penile erection. *Biol. Pharm. Bull.*, **29**: 1383-1386.
- Pribitkin EA (2005). Herbal Medicine and Surgery. *Seminars in Integrative Medicine*, **3**: 17-23.
- Rajendrin V, Joseph T and David J (1996). Plant drugs. *Indian. Drugs*, **33**: 465.
- Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel SW, Herrera-Ruiz D and Torres E (2008). Risks and benefits of commonly used herbal medicines in Mexico. *Toxicol. Appl. Pharmacol.*, **227**(1): 125-135.
- Rowland DL and Tai W (2003). A review of plant-derived and herbal approaches to the treatment of sexual dysfunctions. *J. Sex Marital Ther.*, **29**: 185-205.
- Shappira Z (1987). Preliminary studies on the potential of some indigenous medicinal plants to regulate reproduction in white rats. *Israel Journal of Botany Basic and Applied Plant Sciences*, **36**(4): 212.
- Shukla YN, Srivastava A, Kumar S and Kumar S (1999). Phytotoxic and antimicrobial constituents of *Argyrea speciosa* and *Oenothera biennis*. *J. Ethnopharmacol.*, **67**: 241-245.
- Teng L, Shaw D and Barnes J (2008). Practice of traditional Chinese herbal medicine shops in central London. *Phytochemistry Letters*, **1**: 94-98.
- Visavadiya NP and Narasimhacharya AVR (2007). Hypocholesteremic and antioxidant effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. *Phytomedicine*, **14**: 136-142.
- Wang ZG and Ren J (2002). Current status and future direction of Chinese herbal Medicine. *TRENDS in Pharmacological Sciences*, **23**: 347-348.
- Wong WY, Thomas CMG, Merkus JMW (2000). Male factors subfertility: Possible route causes and the impact of nutritional factors. *Fertil. Steril.*, **73**: 435-442.