

The Effects of Aloe Vera Extract on Spermatogenesis and Reproductive Hormones in Mice

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Abstract: Aloe vera is perennial herbaceous plant of Liliacea family. This plant has many medicinal properties which have made it an important pharmaceutical plant. The effects of hydroalcoholic extract of aloe vera gel on testis tissue and spermatogenesis of mice (Balb/C race) were studied in this study. Samples were divided randomly in 5 groups including control, placebo and 3 treatment groups with 10 members in each group which all groups were kept in similar conditions. Hydro alcoholic extract was prepared in 50, 100 and 200 mg kg⁻¹ doses and was injected in sample's peritoneum every other day for 20 days. Placebo group received only normal saline. Testosterone, LH and FSH hormones levels and also the number of stem cells, primary spermatocytes and relative testis weight were measured. All hormone tests were done using RIA. Results showed that aloe vera increased the number of stem cells and primary spermatocytes significantly in all treatment groups. Also, significant changes occurred in 3rd experimental group (200 mg kg⁻¹). Testosterone was increased in 3rd group ($p < 0.05$) while LH and FSH hormones did not show any significant changes. According to results, aloe vera extract has positive effect on testosterone and histological properties of testis, dose dependently.

Key words: Aloe vera extract, testosterone, stem cells, primary spermatocytes, testis weight

INTRODUCTION

Recent progresses in industry and biotechnology have led to improvements in human life. But, many physical, chemical and biological factors have been produced, also which affect our environment. Considering the sensitivity of reproduction system, it is being easily affected by mentioned factors. Infertility and low fertility are various degrees of reproduction system failures. Infertility has affected about 15% of world couples and 40-50% of infertilities are because of male factors (Singh *et al.*, 2001). Existence of natural and sufficient spermatozooids in sperm is a necessity for fertility. Low fertility of male sex can be because of inability to produce sperm and ejaculation, premature ejaculation, decreased libido and inability or unwillingness for intercross (Hamman, 2008).

Plants have a long time history in medicine and have been main resource for drugs, even in modern world. Pharmaceutical plants are frequently used in developing countries for curing chronic diseases. Even in other countries, plants are really important in drugs

production. On the other hand, many users of chemical drugs tend to use herbal drugs because they are generally safer than synthetic drugs. It is estimated that about 25% of prescription drugs have plant origin. Also, about 75% of used anti cancers from 1981-2006 have been derived from plants (Estakhr and Javdan, 2011). Plant extracts are a mixture of various compounds which enables them to react with several molecular targets. Therefore, micro organism can hardly resist against these compounds (Newman and Cragg, 2007). Even one active matter in a plant has more effect on human body than synthetic type of it. This proves superiority of herbal medicines. In this condition, effective matter of drug is not a chemical compound only but it has physiological balance which body can tolerate it better. Also, it does not have side effect which is a strong reason for preference of natural medicine. Pharmaceutical plants are readily available resources for effective matters which are similar to matters of human body metabolism and are easily absorbable by body (Vinson *et al.*, 2005). Now-a-days, using modern technology in pharmaceutical plants industry has led to diversity in these products and it is expected to eliminate

undesirable chemicals by producing herbal products for various uses in medicine, veterinary and livestock husbandry. One of advantages of pharmaceutical plants and their extract is also curing infertility and low fertility.

Aloe is a cactus-like perennial herbaceous plant which grows easily in arid warm regions of Africa, North America, Europe and Asia. Also, it can be sown as pot plant in houses. Aloe vera is reproduced via seed, leaves cuttings and other parts of origin plant. This plant contains many vitamins, including antioxidant vitamins like A and C vitamins of B group like thiamin, niacin, B2 (Riboflavin), B12 and folic acid. Sodium, potassium, calcium, magnesium, manganese, copper, chrome and iron are found in aloe vera. Gel of this plant has 20 of 22 essential amino acids for human and 7 from 8 necessary amino acids which body cannot produce itself, including: Arginine, asparagin, glutamic acid, aspartic acid and cerin (Vinson *et al.*, 2005).

Aloe vera is a pharmaceutical plant which can be useful for curing various diseases and improving body's physiology. It can be used, as a natural antioxidant with high potential of reducing fats oxidation and oxidative stresses (Vinson *et al.*, 2005). This plant raises natural defense of body against oxidative stresses via increase in amount and activity of natural antioxidant enzymes like liver catalase, superoxide dismutase and glucose-6-phosphate dehydrogenase. Vitamins C and E are important antioxidant factors. Aloe vera can enforce its antioxidant role indirectly via strengthening these vitamins. Researchers have proved that this is done via increase in concentration of these vitamins in blood and also increase in their persistence and activity period in blood (Kooshesh *et al.*, 2007). This plant has also many uses in traditional medicine including: Orally, as a laxative to treat constipation (in patients with anal fissures, hemorrhoids, anorectal surgery) and also for preparation of diagnostic tests for gastrointestinal disease; curing gastric ulcer, tuberculosis, fungal infection and decreasing blood glucose; curing gastric ulcer, tuberculosis and fungal infections and reducing blood glucose levels; topical treatment of inflammatory skin disorders and minor wounds, minor skin irritations, including burns and second degree burns from thermal radiation, bruises and scratches. In the cosmetics industry, as a hydrating liquids, creams, sunscreen lotions, shaving creams, lip creams, healing creams and face masks. As anti bacterial and for curing insect bites.

In this study, the effect of aloe vera extract was examined via inter peritoneum injection. The advantage of

this method is using definite dose of extract. Hormones of pituitary-gonad axis (FSH and LH), testosterone hormone and testis histology were also investigated.

MATERIALS AND METHODS

The 40 male mature mice in weight range of 30 ± 5 g were prepared and kept for 15 days in similar condition. Sample had similar access to food and water, $28-32^{\circ}\text{C}$ temperature and natural light along experiment period. Mice were divided in 5 groups randomly with 8 members in each group. Groups were control, placebo (0.5 cc of normal saline injection per day) and 3 experimental groups: 0.5 cc of extract with 50, 100 and 200 mg kg^{-1} doses every other day for 20 days (Modaresi, 2012). After 20 days blood samples were prepared and LH, FSH and testosterone hormones were measured. Also, testes with epididymis were removed and tissue slides were prepared using hematoxylin-eosin method. Slides were studied using light microscopy and number and arrangement of spermatogenic cells were examined in seminiferous tubes. Researchers tried to select similar stages of spermatogenesis to appropriate comparison. Obtained data were analyzed using SPSS program and means were compared using Duncan multiple ranges test ($p < 0.05$).

RESULTS AND DISCUSSION

Study of basic cell numbers showed significant increase in all 3 experimental groups in proportion to control group ($p < 0.5$). Comparing the number of primary spermatocytes in prepared tissue slides showed that 2nd treatment (100 mg kg^{-1}) and 3rd group (200 mg kg^{-1}) had significant higher spermatocytes ($p < 0.05$) (Fig. 1).

Study of FSH, LH and testosterone hormones showed that there was not significant different between LH and FSH hormones (mIU mL^{-1}) of treatment groups and control but 3rd experiment group (200 mg kg^{-1}) showed significant increase ($p < 0.05$) in testosterone level (Fig. 2).

According to results of counting stem cells and primary spermatocytes, 2nd and 3rd experimental groups had significant differences with control group ($p < 0.05$). One important factor which has probably affected and increased stem cells and primary spermatocytes is anti apoptosis factor in aloe vera extract which increases spermatozoa. Increase in apoptosis of reproductive cells destroys the balance between reproduction and death of these cells and causes impaired spermatogenesis. Bax protein is a member of Bcl-2 family which acts as an apoptosis inducer. Increase in reproductive cells number in seminiferous tubules can be ascribed to increase in

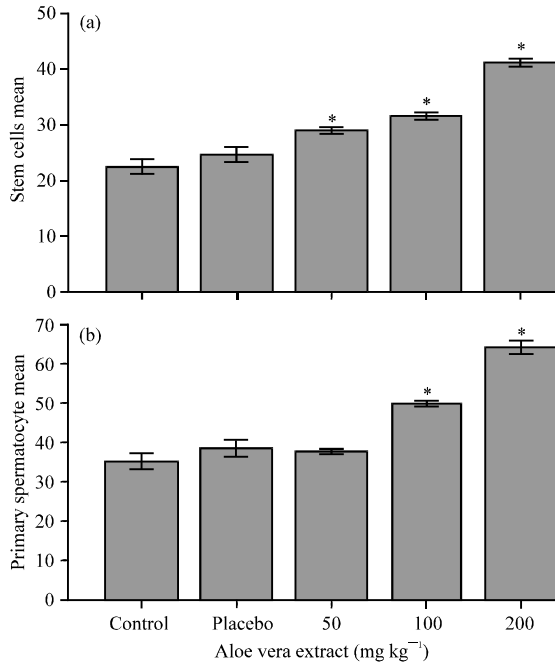


Fig. 1: Stem cell and primary spermatocytes numbers in studied groups

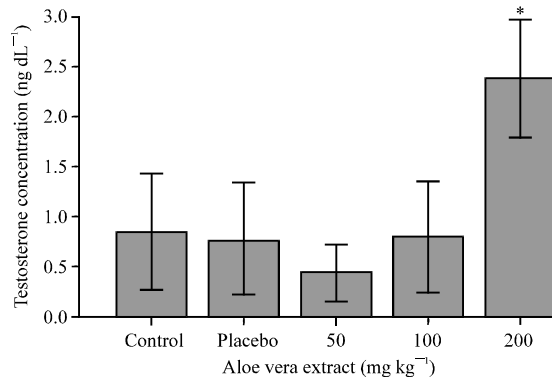


Fig. 2: Testosterone hormone amount of studied groups

reproduction of these cells and reduction in their death (Kooshesh *et al.*, 2007). Cell death occurs in 2 ways: Necrosis and apoptosis. If there is no sign of inflammation and congestion of red blood cells probability of necrosis is very low. If bax protein amount is high apoptosis is probable. Increase in concentration of serum glucocorticoids due to stress can lead to controlling activity of testosterone making enzymes and reduction in leydig cells which cause decrease in testosterone level. Stem cells and primary spermatocytes show the highest apoptosis under androgen deprivation (Lee, 2001). Researches show also undesirable effects of free radicals on reproduction activity and fertility of sperms. These radicals will cause failures in natural activity of cells if

they are not controlled permanently. Immunity from pathological and cytotoxic lesions has positive effect on spermatogenesis trend and transition from germ cells to mature sex cells. Therefore, considering that flavonoides and A, C and E vitamins of aloe vera can reduce free oxygen radicals, it can support sperm production and health of sperm against oxidative stresses (Joseph and Raj, 2010). In a study, about effects of pharmaceutical plants on male sexual system, spermatogenic activity of aloe vera gel and pulp were studied on male mice and it was turned out that this plant and especially its gel have high spermatogenic activity by increasing sperm parameters like sperm numbers and testis weight (Cross *et al.*, 2002).

Testis weight was increased in 3rd experimental group. The reason is increase in amount of stem cells and primary spermatocytes which can be because of direct effects of extract like antioxidant, anti apoptosis, anti mutation effects and also improving epithelial cells. On the whole, the activity of is related to its weight and size are. Therefore, increase in weight has positive effect on spermatogenesis and production of androgen hormone. Meanwhile, increase in testosterone level causes increase in testis weight (Estakhr and Javdan, 2011).

Considering hormone amounts, researchers can conclude that hormone changes did not have effect on HPG axis because although testosterone was increased significantly, there was not any obvious reduction in gonadotropin amount. Extract can affect spermatogenesis directly via affecting germinal cells and stimulating their physiological activity and stimulating cell division. It can also affect indirectly via stimulating leydig cells and increase in testosterone hormone. Incidentally, it is probable that a compound directly affect testis tissue or other parts of sexual system and cause sperm production (Kooshesh *et al.*, 2007). Flavonoides are from phytoestrogens which are natural herbal compounds with an estrogen-like structure. Phytoestrogens can affect sexual hormones. They also have antioxidant, anti-allergic, inflammatory and cancer properties. Some extant flavonoides in aloe vera extract cause increase in testosterone by opposition with enzymes which contribute in testosterone metabolism like aromatase and 5- α reductase. Antioxidants of aloe vera (especially vitamin E) are the most important defense factor against peroxidation of cell membrane phospholipids and have the highest antioxidant biological activity.

Vitamin E prohibits reduction in number of leydig and sertloji cells and increases them. Increase in leydig cells number causes increase in testosterone. Also, this vitamin improves testis weight, seminiferous tubule diameter and thickness of the germinal epithelium (Estakhr and Javdan, 2011).

CONCLUSION

It seems that compounds in aloe vera extract does not affect pituitary-hypothalamus-testis axis and affects spermatogenesis directly via stimulating activity of germinal cells and cell division and also indirectly via stimulating leydig cells and increasing testosterone hormone.

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