

# Synergistic effect of *Aloe vera* and Vitamin C in regulating the steroidogenic and spermatogenic abnormalities of streptozotocin induced male diabetic rats.

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## Abstract

Diabetes mellitus is potentially affecting directly or indirectly, various functions of the reproductive system. *Aloe vera* extract is an effective agent in ameliorating the oxidative stress found in diabetes. Vitamin C, or ascorbic acid, is an important antioxidant substance in biological systems. The purpose of this study was to investigate the synergistic effects of *Aloe vera* extract and Vitamin C on spermatogenic and steroidogenic activities of diabetic induced male rats. *Aloe vera* leaf gel extract, Vitamin C and a mixture of both administered to three groups of male wistar strain albino rats for 30 days. Then the activity levels of 3- $\beta$  HSD, 17- $\beta$  HSD and sperm parameters and the levels of hormones like Testosterone, FSH and LH were analysed. The diabetic rats treated with *Aloe vera* leaf gel extract in combination with Vit.c exhibited increased activity levels of 3- $\beta$  HSD, 17- $\beta$  HSD and androgen dependent enzyme activities when compared to other groups. Analysis of testosterone, FSH and LH levels has shown that the levels these hormones increased in the group that treated with mixture of both *Aloe vera* and Vitamin C. According to the results of this study, the mixture of *Aloe vera* and Vitamin C has good potential in increasing the steroidogenesis and spermatogenesis in diabetic induced male rats.

**Keywords:** Diabetes, Steroidogenesis, Spermatogenesis, *Aloe vera*, Vitamin C, Testosterone, FSH, LH, 3- $\beta$  HSD, 17- $\beta$  HSD.

## Introduction

Diabetes mellitus is the most common metabolic disease in the world and has become a serious problem of modern society due to long-term health associated complications (Coman et al., 2012). It is potentially devastating, expensive, treatable but incurable lifelong disease (Al Attar, 2010). Diabetes mellitus is known to cause many systemic complications such as cardiovascular diseases, hypertension (Diagnosis and Classification of Diabetes mellitus, in diabetes care, 2013) and neuropathy. Moreover, male reproductive alterations have also been widely reported in individuals with Diabetes mellitus (Ballester et al., 2004). Numerous studies in male diabetic individuals have demonstrated a marked reduction in fecundity (Scarano et al., 2006; Cameron et al., 1990), as well as impairment of sperm quality (Scarano et al., 2006; Amaral et al., 2006) and higher percentage of spermatozoa with nuclear DNA damage (Agbaje et al., 2007). Diabetes mellitus induced effects on testicular function have been attributed to the lack of insulin (Ballester et al., 2004), which is the leading hormone responsible glucose homeostasis regulation (Bogan, 2012). Diabetes mellitus is potentially affecting directly or indirectly, various functions of the reproductive system due to vascularization and endothelial dysfunction (Brownlee, 2005; Jackson, 2004; Glenn, 2003). About 90% of the diabetic male patients have disturbances in sexual function including a decrease in libido, impotence and infertility (Feng et al., 2001). Many of the already existing drugs to manage Diabetes mellitus fail as a curative agent for diabetic complications and also have a number of serious adverse effects that can discourage patient compliance (Vasconcelos et al., 2011). Over the years, the use of medicinal plants has become a feasible alternative for the treatment of Diabetes mellitus or to reinforce the currently used treatments (Coman et al., 2012; Hays et al., 2008). *Aloe vera L.* is one of the medicinal plants which is a traditionally well acknowledged plant in the management of diabetes. In the male reproductive system, vitamin C is known to protect spermatogenesis and it plays a major role in semen integrity and fertility both in men [Agarwal and Prabhakaran, 2005; Eskenazi et al., 2005] and animals, increases testosterone levels [Sonmez et al., 2005] and prevents sperm agglutination. The present study is aimed to assess the effect of *Aloe vera* gel in combination with vitamin C in regulating the abnormalities induced by diabetes mellitus in male reproductive system of albino rats in particular.

## Materials and Methods

### Preparation of *Aloe vera* extract

*Aloe vera* extract was prepared from *Aloe vera* leaf gel according to the published procedure (Grive et al., 1975), with slight modifications. The fleshy solid gel in the center of the leaf was scratched with spoon, collected, homogenized and lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator at 60°C. The residue was stored in dry sterilized small containers at 4°C till further use.

### Preparation of vitamin C

Vitamin C (L-Ascorbic acid; SIGMA-ALDRICH, St. Louis, MO, USA) was prepared daily by diluting the required quantity in the corresponding volume of warm water and stored in a dark container to protect against light.

### Selection of animals

Male albino Wistar rats (180 ± 20 g) were obtained from the Indian Institute of Science, Bangalore, India. Animals were housed in clean polypropylene cages maintained under a 12 h: 12 h schedule of light: dark cycle at 25 ± 2°C with a relative humidity of 50 ± 5 %. The animals were fed on pellet diet (manufactured by Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. This study was carried out according to guidelines for the care and use of laboratory animals.

### Induction of experimental Diabetes

After fasting, diabetes was induced by intraperitoneal injection of single dose STZ (Sigma, St. Louis, Mo., USA) freshly dissolved in 0.1 M cold sodium citrate buffer, (pH 4.5) at a dose of 40 mg/kg body weight (Bunyaphatsara et al., 1996). After injection, they had a free access to food and water. 5% glucose solution was given to drink overnight to counter hypoglycaemic shock. The animals were considered as diabetic, if their blood glucose (Accu chek sensor comfort glucometer (manufacture - Johnson and Johnson) levels were above 250 mg/dl on the 4th day after STZ injection.

### Experimental design

Rats were randomly divided into five groups of six animals in each group.

Group –I: Control rats.

Group –II: Diabetic control rats (40mg/kg bodyweight of STZ)

Group –III: Diabetic+ *Aloe vera* extract (300 mg/kg body weight in ethanol solution daily once in a day by an intragastric tube for 30 days).

Group– IV: Diabetic+ Vitamin C (150 mg/kg body weight in solution daily once in a day by an intragastric tube for 30 days).

Group– V: Diabetic+ *Aloe vera* extract (300 mg/kg body weight in ethanol solution) + Vitamin C (150 mg/kg bodyweight in solution) daily once in a day by an intragastric tube for 30 days).

The body weights of control and experimental groups were recorded at an interval of one week till the completion of the experimental period (30 days). The blood glucose levels were carried out by using Accu Chek glucometer (Manufacture: Johnson and Johnson) every week during experimental period. The animals were sacrificed after 24hrs of the last treatment (30th day) by cervical dislocation and the tissues like testes, epididymis, seminal vesicles and prostate gland were isolated. The tissues were washed with ice-cold saline, and immediately stored in deep freeze at -80°C for biochemical analysis and enzymatic assays.

## Hormonal assay

Blood samples were collected from abdominal aorta, separated after centrifugation (3000 rpm) and stored at  $-80^{\circ}\text{C}$ , to carry out the hormonal assays. Hormones such as testosterone, FSH and LH levels were measured by radioimmunoassay coat-A-count kit (diagnostic products corporation, LA, Calif) using Packard Cobra gamma-counter.

## Enzymatic assay

The activities of steroidogenic marker enzymes like  $3\beta$ - Hydroxy Steroid Dehydrogenase ( $3\beta$  HSD) and  $17\beta$ - Hydroxy Steroid Dehydrogenase ( $17\beta$  HSD) were studied in the testis.

## Sperm collection

To investigate the quality of the sperms, caudal part of epididymis was sliced in the buffer T6 and were put in incubation system for 10 minutes, then a drop of buffer containing sperms was taken to be mounted on slide and the percentage of sperm motility (fast and slow) and also the percentage of non-motile sperm were counted. After that, 0.5 ml of buffer containing sperm was diluted by the white measuring pipette with proportion of 1/10 and using neubauer slide, the number of sperms were counted and multiplied by ( $\times 10^6/\text{mm}^3$ ). The obtained data were analyzed using SPSS software program and One-way ANOVA test.

## Statistical analysis

The data were statistically analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's *t*-test and 'p' value  $<0.05$  was considered significant. The data were presented as mean  $\pm$  S.D. and analysis was carried out by using SPSS 16.0.1 program.

## Results

**Hormone analysis:** the results shown that the serum testosterone levels were significantly decreased in STZ induced diabetic rats (-80.60) when compared to control rats. The Diabetic rats treated with mixture of *Aloe vera* and Vit.C have shown the significant increased levels of testosterone when compared to other diabetic rats treated with *Aloe vera* and Vit.C separately. The same trends were observed with FSH and LH (Table 1).

**Steroidogenic marker Enzyme activities:** The activity levels of  $3\beta$  -HSD and  $17\beta$ - HSD were significantly ( $p<0.05$ ) decreased in STZ induced diabetic rats when compared to control rats. But diabetic rats treated with mixture of *Aloe vera* and Vit.C have shown the increased activity levels of steroidogenic marker enzymes like  $3\beta$  -HSD and  $17\beta$ - HSD when compared to other groups of treated diabetic rats (Table-2).

**Sperm analysis:** STZ induced diabetes caused a significant reduction in sperm count and motility, but increased the abnormal sperm cell percentage. In Diabetic rats treated with the mixture of *Aloe vera* and Vit.C, the sperm count and motility were significantly increased; abnormal sperm cell percentage was decreased when compared to diabetic rats treated with *Aloe vera* and Vit.C separately (Table-3).

**Table-1: Hormone Analysis**

Hormone	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Testosterone (ng/mL)	1.65 <sup>a</sup>	0.32 <sup>b</sup> $\pm 0.01$ (-80.60)	0.67 <sup>b</sup> $\pm 0.02$ (-59.39)	0.48 <sup>ab</sup> $\pm 0.01$ (-70.90)	0.93 <sup>b</sup> $\pm 0.03$ (-43.63)
LH (ng/mL)	6.60 <sup>a</sup>	3.03 <sup>b</sup> $\pm 0.02$ (-54.09)	4.02 <sup>b</sup> $\pm 0.03$ (-39.09)	3.60 <sup>ab</sup> $\pm 0.01$ (-45.45)	4.95 <sup>b</sup> $\pm 0.02$ (-25.0)
FSH	6.70 <sup>a</sup>	5.30 <sup>a</sup>	5.76 <sup>a</sup>	5.62 <sup>a</sup>	5.91 <sup>b</sup>

(ng/mL)		±0.01 (-20.89)	±0.03 (-14.02)	±0.02 (-16.11)	±0.03 (-11.79)
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Values are mean ± S.D. of 6 individuals

Values in the parentheses are percent change from the control.

Mean values in a row that do not share the same superscript differ significantly at p<0.05.

**Table-2 : Steroidogenic potential of the testis by studying marker enzymes**

Parameter	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Cholesterol (mg/g fresh tissue)	3.185 <sup>a</sup> ±0.91	4.12 <sup>b</sup> ±0.82 (29.35)	3.96 <sup>b</sup> ±0.74 (24.33)	3.84 <sup>b</sup> ±0.72 (20.56)	3.42 <sup>a</sup> ±0.81 (7.37)
3 β –HSD (μ moles of NAD <sup>+</sup> reduced/mg protein/ min.)	0.382 <sup>a</sup> ±0.14	0.234 <sup>b</sup> ±0.12 (-38.74)	0.264 <sup>b</sup> ±0.21 (-30.89)	0.218 <sup>b</sup> ±0.16 (-42.93)	0.314 <sup>a</sup> ±0.23 (-17.80)
17 β- HSD (μ moles of NADPH oxidised/mg protein/ min.)	0.565 <sup>a</sup> ±0.37	0.276 <sup>b</sup> ±0.187 (-51.15)	0.312 <sup>b</sup> ±0.32 (-44.77)	0.297 <sup>b</sup> ±0.28 (-47.43)	0.421 <sup>b</sup> ±0.31 (-25.48)

Values are mean ± S.D. of 6 individuals

Values in the parentheses are percent change from the control.

Mean values in a row that do not share the same superscript differ significantly at p<0.05.

**Sperm Analysis (Table-3)**

Parameter		Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Sperm count		171.56 <sup>a</sup> ±13.82	134.21 <sup>b</sup> ±5.12 (-21.77)	153.13 <sup>a</sup> ±6.21 (-10.74)	143.34 <sup>a</sup> ±7.14 (-16.44)	163.24 <sup>a</sup> ±6.32 (-4.84)
Sperm Motility %	Fast	13.76 <sup>a</sup> ±2.82	3.7 <sup>b</sup> ±1.21 (-73.11)	7.2 <sup>b</sup> ±2.38 (-47.67)	6.1 <sup>b</sup> ±2.45 (-55.66)	10.2 <sup>b</sup> ±2.57 (-25.87)
	Slow	37.21 <sup>a</sup> ±2.04	13.52 <sup>b</sup> ±2.75 (-63.66)	24.48 <sup>b</sup> ±1.24 (-34.21)	18.5 <sup>b</sup> ±2.21 (-50.28)	28.32 <sup>b</sup> ±2.34 (-23.89)
	Non-motile	49.02 <sup>a</sup> ±4.56	82.78 <sup>b</sup> ±3.27 (68.86)	68.31 <sup>b</sup> ±3.41 (39.35)	75.3 <sup>b</sup> ±2.37 (53.61)	61.48 <sup>b</sup> ±3.65 (25.41)
Morphology of sperm %	Normal	96.72 <sup>a</sup> ±0.24	95.48 <sup>a</sup> ±0.32 (-1.28)	96.10 <sup>b</sup> ±0.34 (-0.64)	91.23 <sup>b</sup> ±0.27 (-5.67)	96.51 <sup>a</sup> ±0.31 (-0.21)
	Abnormal	3.28 <sup>a</sup> ±0.21	4.52 <sup>b</sup> ±0.31 (37.80)	3.9 <sup>b</sup> ±0.32 (18.90)	3.77 <sup>b</sup> ±0.34 (14.93)	3.49 <sup>a</sup> ±0.32 (6.40)

Values are mean ± S.D. of 6 individuals

Values in the parentheses are percent change from the control.

Mean values in a row that do not share the same superscript differ significantly at  $p < 0.05$ .

## Discussion

Diabetes mellitus is a cause of male sexual dysfunction, which by itself may contribute to sub fertility or even infertility (Agbaje et al., 2007). Decreased testosterone levels in diabetic induced rats suggesting a decrease in the function of Leydig cells which might be caused by reduction in insulin secretion (Ballester et al., 2004). The treatment with antioxidants had protective effect on testicular oxidative damage and germ cell apoptosis (Mohassab et al., 2011). It is known that vitamin C plays key roles in the synthesis of testosterone (Sonmez et al., 2005) and in our study vitamin C-treated hyperglycemic rats showed partial recovery of testosterone level. Moreover Aloe vera extract and Vitamin C up regulate steroidogenic enzymes in testis, thereby increasing testosterone levels in diabetic induced rats treated with Aloe vera extract in combination with Vitamin C. This is inconsonance with the results obtained in this study with regard to activity levels of steroidogenic marker enzymes. The marker enzymes of steroidogenesis such as  $3\beta$  HSD and  $17\beta$  HSD were inhibited in the testis of diabetic induced rats confirming the possible derangement in the testicular steroidogenesis. The activity level of  $3\beta$  HSD had been significantly decreased in diabetic induced rats in comparison to control. Since  $3\beta$  HSD mobilizes cholesterol towards the formation of pregnenolone, a precursor for the androgenesis. Similarly the activity levels of  $17\beta$  HSD, which mobilizes pregnenolone towards the testosterone formation also decreased in diabetic induced rats. The activity levels of these two enzymes significantly increased in the testis of hyperglycemic rats treated with the combination of Aloe vera extract and Vitamin C when compared to other groups of rats.

In the present study, the reduced LH level corroborates previous studies that reported diminished LH release from pituitary gland in hyperglycemic male rats (Olivares et al., 2009). The partial recovery of LH levels in the vitamin C treated group may be related to the fact that ascorbic acid can be a vitaminergic transmitter that activates the release of LH and FSH from the anterior pituitary gland (Karanth et al., 2001). As in earlier reports (Steger et al., 1989, Abou self et al., 2001), in the present study also the plasma levels of FSH were also reduced in hyperglycemic rats. The LH and FSH levels were recovered significantly in the diabetic rats treated with Aloe vera extract in combination with Vitamin C.

Hyperglycemic rats showed reduced sperm count (Hassan et al., 1993), impairment in sperm motility and morphology (Navarro-Casado et al., 2010). In the present study, reduced sperm count and increased abnormal sperm cell percentage were observed in diabetic induced rats, but these abnormalities were significantly attenuated by the combined treatment of Aloe vera extract and vitamin C. This result may suggest that vitamin C could have an effect on spermiogenesis process and it could favor, at least partially, normal sperm production. It is known that vitamin C increases gamete mobility since it reduces oxidative stress level (Hsu et al., 1998). Diabetes increases the thickness of the basement membrane and causes the decrease in the amount of producing sperm. In addition the decrease in the number of Sertoli cells results in the decrease in spermatogonia cells (JafariBarmak et al, 2013). Guneli et al. in a study, indicate that diabetes causes testicular tissue changes through creating cell death (Apoptosis), the increase in the thickness of testicular capsule, the atrophy of seminiferous tubules, the reduction in tubule diameter and the decrease in the somatic Leydig and Sertoli cells (Sonmez et al., 2005.). Kiani (2011) in a study indicated that the structural changes in the diabetic rats testicular tissue are not because of the streptozotocin related to the side effects of this composition, but the effects of diabetes on the testicular performance are due to the insufficient insulin production and consequently the decrease in the effect of this hormone in the regulation of Sertoli, Leydig and spermatogonia cells activities. In a study done by Rajasekaran et al. (2004) indicates that *Aloe vera* moderates the level of hepatic glycogen by decreasing the activity of glycogen phosphorylase and increasing the activity of glycogen synthetase. Pari et al. (2000, 2002) indicate that probably *Aloe Vera* does the hypoglycemia operation by the potential of insulin release from the beta cells of the islets of Langerhans or insulin release from the bound form.

## Conclusion

In conclusion, the present study showed that combined supplementation of *Aloe vera* extract and vitamin C minimized some alterations in the male reproductive system caused by hyperglycemia such as reduction of testosterone, LH and FSH levels and impairment in sperm morphology. Combination of *Aloe vera* extract and Vitamin C might be effective in attenuating the abnormalities in male reproductive system caused by Diabetes mellitus. Therefore it can be concluded that combination of *Aloe vera* and Vitamin C is helpful than *Aloe vera* and Vitamin C separately in the treatment of Diabetes

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