Effect of Combination of Aloe vera and Vitamin C on Androgen Dependent Enzyme Activities of Reproductive Tissues in Streptozotocin Induced Diabetic Male Rats

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Abstract

Diabetes has been associated with reproductive impairment in both men and women, and its impact on reproduction can be profound, as seen by diminution in fertility and increase in reproductive losses. Hyperglycemia-induced oxidative stress has been reported to be associated with testicular failure leading to sexual dysfunction, impotence and infertility. *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 activities of androgen dependent enzymes in diabetic induced male rats. *Aloe vera* leaf gel extract, Vitamin C and a mixture of both administered to three groups of diabetic male wistar strain albino rats for 30 days. Then the activity levels of androgen dependent enzymes like SDH, ACP, ALP, AlAT and AAT were studied in testis, epididymis, prostate gland and seminal vesicles. The serum testosterone levels were also studied in all the groups of experimental animals. The diabetic rats treated with *Aloe vera* leaf gel extract in combination with Vitamin C exhibited increased activity levels of androgen dependent enzyme activities and increased testosterone levels when compared to other groups. These results showed that the combination of *Aloe vera* and Vitamin C has good potential in increasing the activity levels of androgen dependent enzymes in diabetic induced male rats.

Keywords: Diabetes Mellitus, *Aloe vera*, Vitamin C, SDH, ACP, ALP, Al AT, AAT.

Introduction

Diabetes has been associated with reproductive impairment in both men and women (Baccetti et al., 2002) and its impact on reproduction can be profound, as seen by diminution in fertility and increase in reproductive losses. About 90% of the diabetic male patients have disturbances in sexual function including a decrease in libido, impotence and infertility (Feng et al., 2001). Male reproductive alterations have also been widely reported in individuals with Diabetes mellitus (Ballester al., 2004). Diabetes induces testicular alterations, disrupting the metabolic cooperation between the cellular constituents of blood testis barrier, with dramatic consequences on sperm quality and fertility (Alves et al., 2013). Its induced effects on testicular function have been attributed to the lack of insulin (Ballester et al., 2004), which is the leading hormone responsible for glucose homeostasis regulation (Bogan, 2012). The prostate health may contribute to male diabetic infertility since the volume of prostatic secretions may reduce during prostate gland pathology (Weidner *et al.,* 1999). Several studies have reported direct relationship between diabetes and prostate pathologies (Bansal *et al.,* 2013), which may be a consequence of metabolic aberrations and alterations in sex hormone levels in diabetic patients (Bonovas *et al.,* 2004). Moreover, pathologies of prostate, triggered by decreased insulin secretion or resistance to insulin action (Meyer *et al.,* 2000), may lead to decrease in prostatic secretions (Marconi *et al.,* 2009). These factors therefore play significant role in prostate functions, from enzymatic activity to sperm motility. Neuropathy, one of the usual complications of diabetes, has a negative effect on the function of the seminal vesicles. This functional impairment of the seminal vesicles further affects the sexual function and the fertility potential of the male (Ali et al., 1993).The findings of P Tsounapi *et al* (2016) suggested that diabetes-induced oxidative stress mediates probably a secondary mechanism to damage the seminal vesicles function.

Many plants extracts and products were shown to possess significant antioxidant activity (Sabu and Kuttan,2002). *Aloe vera* is an ornamental and medicinal plant. It is being used therapeutically, since Roman times and perhaps long before. In the past 25 years there have been reports on the antidiabetic activity of *Aloe vera* extracts (Rajasekaran et al., 2005). Small molecule antioxidants such as vitamin C, Vitamin E also play important

roles as cellular antioxidants. 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 (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 combined effect of *Aloe vera* extract and vitamin C on the activity levels of androgen dependent enzymes in the reproductive tissues of diabetic induced male rats.

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 ± 2ºC 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 (Bunyapraphatsara 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, prostate gland and seminal vesicles were isolated. The tissues were washed with ice-cold saline, and immediately stored in deep freeze at -80^{0}C for biochemical analysis and enzymatic assays.

Testosterone Assay

Blood samples were collected from abdominal aorta, separated after centrifugation (3000 rpm) and stored at -80^0 C, to carry out the hormonal assays. The testosterone was measured by radioimmunoassay coat-A-count kit (diagnostic products corporation, LA, Calif) using Packard Cobra gamma-counter.

Determination of acid and alkaline phosphatase activities:

The activity of Acid phosphatase (ACP) was determined by the method of Fishman and Lerner (1953). Alkaline phosphatase (ALP) activity was measured spectrophotometrically by monitoring the concentration of phenol formed when ALP reacts with disodium phenyl phosphate at 680 nm as described by Williamson (1972).

Alanine and aspartate aminotransferases activities determination:

Alanine aminotransferase (ALT) and Aspartate aminotransferases (AAT) activities were determined by using the methods of Reitman and Frankel (1957).

Statistical analysis

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

Results

The data in Table 1 indicates the decreased testosterone level in diabetic rats (-80.60%) over the control and the same is significantly recovered in diabetic rats treated with combination of *Aloe vera* extract and vitamin C when compared to other treated groups. The data in tables 2-5 indicate a remarkable decrease in the activity levels of androgen dependent enzymes in testis, epididymis, prostate gland and seminal vesicles in diabetic induced rats over the controls. The activity levels of these enzymes were more increased in the diabetic rats which were given combined treatment of *Aloe vera* extract and Vitamin C than the diabetic rats treated with **Aloe vera** extract and vitamin C separately.

Table -1: Hormone Assay

Hormone	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Testosterone (ng/mL)	1.65^{a}	0.32^{b} ±0.01 (-80.60)	0.67^{b} ±0.02 (-59.39)	0.48^{ab} ±0.01 (-70.90)	0.93^{b} ±0.03 (-43.63)

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: Androgen dependent enzyme activities in Testis

Parameter	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Sorbitol dehydrogenase (µ moles of formazan formed/mg protein/h)	2.14[a] ±0.724	1.24[b] ±0.862 (-42.05)	1.52[a] ±0.82 (-28.97)	1.34[a] ±0.79 (-37.38)	1.76[ab] ±0.81 (-17.75)
Acid phosphatase (µ moles of pi formed /mg protein/h)	10.18[a] ±1.14	6.74[b] ±0.91 (-33.79)	7.65[a] ±0.95 (-24.85)	7.01[b] ±0.97 (-31.13)	8.24[ab] ±0.92 (-19.05)
Alkaline phosphatase (µ moles of pi formed /mg protein/h)	61.15[a] ±1.34	41.12[b] ±1.24 (-32.75)	49.21[a] ±1.12 (-19.52)	44.32[a] ±1.14 (-27.52)	53.21[a] ±1.16 (-12.98)
Alanine amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.968[a] ±1.06	0.612[b] ±0.98 (-36.77)	0.713[b] ±0.96 (-26.34)	0.642[a] ±0.95 (-33.37)	0.784[a] ±0.93 (-19.00)
Aspartate amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.769[a] ±0.042	0.513[b] ±0.037 (-33.28)	0.612[b] ±0.039 (-20.41)	0.562[b] ±0.034 (-26.91)	0.681 ±0.041 (-11.44)

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 - 3: Androgen dependent enzyme activities in Epididymis.

Parameter	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Sorbitol dehydrogenase (µ moles of formazan formed/mg protein/h)	1.28 [a] ±0.72	0.824 [b] ±0.862 (-35.62)	0.96 [b] ±0.82 (-25.0)	0.89 [b] ±0.79 (-30.46)	1.01 [b] ±0.81 (-21.09)
Acid phosphatase (µ moles of pi formed /mg protein/h)	14.91 [a] ±0.084	11.21 [b] ±0.91 (-24.81)	12.14 [a] ±0.95 (-18.57)	11.78 [b] ±0.97 (-20.99)	13.12 [a] ±0.92 (-12.00)
Alkaline phosphatase (µ moles of pi formed /mg protein/h)	18.31 [a] ±1.65	14.26 [b] ±1.24 (-22.11)	15.94 [a] ±1.12 (-12.94)	15.01 [a] ±1.14 (-18.02)	16.23 [a] ±1.16 (-11.35)
Alanine amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	1.003 [a] ±0.051	0.627 [b] ±0.98 (-37.48)	0.721 [b] ±0.96 (-28.11)	0.651 [b] ±0.95 (-35.09)	0.812 [b] ±0.93 (-19.04)
Aspartate amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.951 [a] ±0.042	0.491 [b] ±0.037 (-48.37)	0.623 [b] ±0.039 (-34.49)	0.571 [b] ±0.034 (-39.95)	0.712 [b] ±0.041 (-25.13)

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- 4: Androgen dependent enzyme activities in Prostate gland.

Parameter	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Sorbitol dehydrogenase (µ moles of formazan formed/mg protein/h)	0.378 [a] ±0.72	0.24 [b] ±0.862 (-36.50)	0.281 [b] ±0.82 (-25.66)	0.262 [b] ±0.79 (-30.68)	0.314 [a] ±0.81 (-16.93)
Acid phosphatase (µ moles of pi formed /mg protein/h)	20.39 [a] ±0.084	10.91 [b] ±0.91 (-46.49)	12.54 [b] ±0.95 (-38.49)	11.82 [b] ±0.97 (-42.03)	14.26 [b] ±0.92 (-30.06)
Alkaline phosphatase (µ moles of pi formed /mg protein/h)	81.63 [a] ±1.65	74.12 [a] ±1.24 (-9.20)	76.15 [a] ±1.12 (-6.71)	75.01 [a] ±1.14 (-8.10)	77.28 [a] ±1.16 (-5.32)
Alanine amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.88 [a] ±0.051	0.542 [b] ±0.98 (-38.40)	0.67 [b] ±0.96 (-23.86)	0.59 [b] ±0.95 (-32.95)	0.71 [b] ±0.93 (-19.31)
Aspartate amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.722 [a] ±0.042	0.571 [b] ±0.037 (-20.91)	0.631 [a] ±0.039 (-12.60)	0.592 [b] ±0.034 (-18.00)	0.691 [a] ±0.041 (-4.29)

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-5: Androgen dependent enzyme activities in Seminal vesicles.

Parameter	Normal control	Diabetic Control	Diabetic + Aloe vera	Diabetic + Vitamin C	Diabetic + Aloe vera+ Vitamin C
Sorbitol dehydrogenase (µ moles of formazan formed/mg protein/h)	0.516 [a] ±0.72	0.281 [b] ±0.862 (-45.54)	0.312 [b] ±0.82 (-39.53)	0.297 [b] ±0.79 (-42.44)	0.394 [b] ±0.81 (-23.64)
Acid phosphatase (µ moles of pi formed /mg protein/h)	18.18 [a] ±0.084	13.45 [b] ±0.91 (-26.01)	14.68 [b] ±0.95 (-19.25)	14.12 [b] ±0.97 (-22.33)	16.10 [a] ±0.92 (-11.44)
Alkaline phosphatase (µ moles of pi formed /mg protein/h)	62.13 [a] ±1.65	57.13 [a] ±1.24 (-8.04)	58.31 [a] ±1.12 (-6.14)	57.89 [a] ±1.14 (-6.82)	59.64 [a] ±1.16 (-4.00)
Alanine amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.782 [a] ±0.051	0.498 [b] ±0.98 (-36.31)	0.541 [b] ±0.96 (-30.81)	0.511 [b] ±0.95 (-34.65)	0.613 [b] ±0.93 (-21.61)
Aspartate amino transferase (µ moles of sodium pyruvate formed /mg protein/h)	0.661 [a] ±0.042	0.474 [b] ±0.037 (-28.29)	0.523 [b] ±0.039 (-20.87)	0.496 [b] ±0.034 (-24.96)	0.592 [a] ±0.041 (-10.43)

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 considered to produce erectile dysfunction, retrograde ejaculation and reduced levels of testicular hormone and seminal quality changes (De et al., 2016; Al-Roujeaie et al., 2016). It is now known that Diabetes has detrimental effects on male fertility and testis has been the point of emphasis (Abdelali et al., 2016). The levels of reproductive hormones; LH, FSH and testosterone were reduced in diabetic induced rats (Kianifard et al., 2013). Cai et al., (2000) reported that serum testosterone impairment may be linked to varying degrees of testicular and epididymal structural lesions caused by STZ-induced Diabetes. Decreased levels of testosterone in diabetic rats might be due to the suppression of Leydig cell activity and also due to low LH levels in diabetic rats (Kianifard et al., 2013). A recent report suggests that increased production of free radicals causes apoptotic death of the testicular cells under diabetic condition (Kanter et al., 2013).

Decreased testosterone production, on the other hand, may inhibit development of male sex accessories, including growth of the prostate gland (Gilad et al., 1998). The testicular tissue showed conspicuous inhibition in the activity levels of androgen dependent enzymes under induced diabetes condition over that of control testis. The

enzymes such as Sorbitol Dehydrogenase (SDH), Acid Phosphatase (ACP), Alkaline Phosphatase (AlP), Alanine Amino Transferase (Al AT) and Aspartate Amino Transferases (AAT) have shown to be androgen dependent (Davison and Langslow, 1975) and thereby they form the marker enzymes of circulating androgens in the body (Tenniswood et al., 1976). SDH is an enzyme, catalyses the oxidation–reduction reaction, involving the interconversion of fructose and sorbitol. Since SDH is a marker enzyme activating the process of spermatogenesis, such a decrease in the activity of this enzyme in testis under diabetes indicates the possibility of suppressed spermatogenesis. Similarly the activity levels of other androgen dependent enzymes like ACP, AlP, AlAT and AAT also decreased in testis of diabetic induced rats.

The epididymis forms an important site of sperm maturation and storage. In epididymis of diabetic induced rats, decreased activity levels of androgen dependent enzymes indicate the impairment in the functioning of this tissue. The accessory glands such as prostate gland and seminal vesicles also revealed similar pattern of suppressed function in the diabetic induced rats probably due to decreased circulation of testosterone in the body. These results indicate that diabetes was inducing dysfunctions at testicular as well as sex accessory tissue levels, suggesting the inhibition in the function of reproductive organs , chiefly owing to decreased impact of androgens on the tissue metabolism.

The present study demonstrated that after the 30 days of treatment of diabetic rats with *Aloe vera* extract and Vitamin C combination had significant effect on testosterone level and activity levels androgen dependent enzymes in reproductive tissues. More recovery of these enzyme activities occurred in the diabetic rats treated with combination of *Aloe vera* and vitamin C. It was discovered that the administration of *Aloe vera* extract tend to bring the blood glucose levels of diabetic rats towards the normal level (Saif-Ur-Rehman et al*.,* 2011*).* The results are highly correlated with the work of Eman *et al*., 2003 who reported that the aqueous bark extract of *Aloe vera* exhibited effective antihyperglycemic action in diabetic and normal rats. Significant effect of *Aloe vera* on the blood glucose levels of rats is also in agreement with the work of Ayse *et al*., 2004. 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 (Eskenazi et al., 2005) and animals, increases testosterone levels (Sonmez et al., 2005) and prevents sperm agglutination. It is an important chain-breaking antioxidant, contributing up to 65 percent of the total antioxidant capacity of seminal plasma found intracellulary and extracellulary (Makker et al., 2009). Shrilatha and Muralidhara, 2007 reported the protective effect of vitamin C on testicular oxidative stress, sperm oxidative stress and genotoxic effects using a diabetic mice model. Hence combination of *Aloe vera* extract and vitamin C showed a significant improvement in the testosterone level and activity levels of androgen dependent enzymes in the reproductive tissues like testis, epididymis, prostate gland and seminal vesicles in diabetic induced rats..

Conclusion

The *Aloe vera* extract and vitamin C combination had showed a significant effect in increasing the serum testosterone level and activity levels of androgen dependent enzymes in the reproductive tissues like testis, epididymis, prostate gland and seminal vesicles of diabetic induced rats. Hence it can be concluded that due to their hypoglycaemic, spermatogenic and antioxidant properties, *Aloe vera* and Vitamin C combination may have effective therapeutic usage in regulating the reproductive abnormalities in diabetes.

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