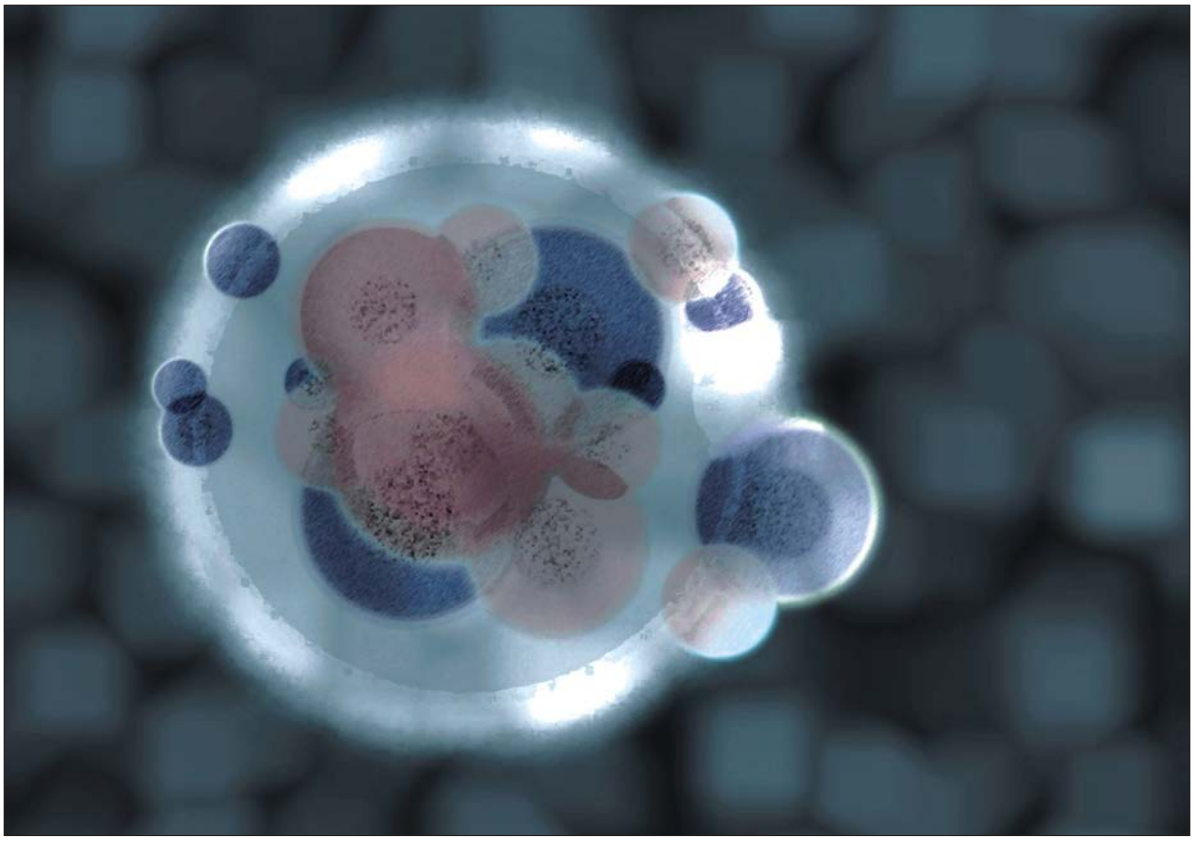

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Impact of Sildenafil Citrate (Viagra) with Ethanol Modulates on Lipid and Lipoprotein in Testis of Albino Rats

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Abstract: Sildenafil citrate (Viagra) is the pharmacological agent used to treat erectile dysfunction in men, a common problem that in the United State affects between 10 and 30 million men. Because this drug has a vasodilatory effect. Sildenafil citrate and ethanol consumption are used in societies world wide and have been identified as injurious to human health. The aim of this study was to evaluate the effects of Sildenafil citrate and Ethanol consumption on lipid and lipoprotein levels in testis tissue and serum of Albino rats. Male Albino rats were divided into eight groups of six animals each and maximum treated for 45 days as follows, control rats were administered with normal saline orally. Sildenafil citrate (1 μg gm^{-1}) and 18% ethanol (5 g kg^{-1} body weight) was given orally at a single doses (short-term) after 1, 2½, 4 and 24 h were sacrificed and 15, 30 and 45 days daily continuous doses (long-term) of drug and ethanol with a single dosage were given and to be sacrificed after 4 h of the last dosage. Further, the average total body weight gain was significantly higher in 30 days treatment, but 45 days no significant change in the body weight of the rats were observed due to the productive role of Sildenafil citrate and ethanol. This combination was found to be increased serum cholesterol, triglycerides, LDL and VLDL levels, whereas the levels of serum HDL was found to be decreased as compared with the control rats. Simultaneously tissue cholesterol and triglycerides significantly ($p<0.05$) inhibited were found to be the rise in lipid and lipoprotein concentrations. Whereas, It is suggested that prolonged exposure to Sildenafil citrate and ethanol administration to rat is found to be increased in lipid and lipoprotein concentrations significantly in an animals.

Key words: Sildenafil citrate (Viagra), ethanol, rat, testis, lipid and lipoprotein

INTRODUCTION

Sildenafil citrate (Viagra) is the agent approved for treatment of erectile dysfunction in men (Kloner and Jarow, 1999). Within the body the drug acts by selective inhibition of cyclic guanosine monophosphate (cGMP), phosphodiesterase type (5), which facilitates the relaxation of smooth muscles and an influx of blood to the corpus cavernosum. The production of these conditions typically results in erection during sexual stimulation. In fact, the regulation of intracellular concentrations of cGMP, PDE 5 was important for relaxation of vascular smooth muscle cells, including those of the penis. Up to now PDE 5 inhibitors acts specifically on penile blood flow and less well in general circulation has not been completely understood. In addition, it is unclear the enhancement of intracellular cGMP concentration does not generate major unwanted biological effects in tissues other than Corpora Cavernosa (CC) (Morelli *et al*., 2004). One possibility is that sexual stimulation, which is necessary for PDE 5 inhibitors effectiveness, causes a specific release of NO in the penis, which would produce a large increase in cGMP synthesis mainly in the tissue (Corbin and Francis, 1999). An alternative possibility is that PDE 5 is not equally distributed in human tissues. Moreover, the presence of a consensus sequence for the androgen receptor in the 5-flanking region of the PDE 5 promoter (Lin *et al*., 2001) suggest that androgens could regulate PDE 5 expression.

Ethanol is a powerful inducer of hyperlipidemia both in animals and humans (Day *et al*., 1993). Recent data have shown beneficial effects of moderate drinking on the risk for cardiac disease(Sillanaukee *et al*., 2000). Ethanol also causes changes in the metabolism of lipoprotein (Hirayama *et al*., 1998). An increase in circulating triglycerides can be produced in fasting individuals after ingestion of ethanol for several hours (Verdy and Gattereau, 1998), as well as during administration of

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ethanol containing diets for several days (Schapiro *et al*., 1965). The accumulation of fat in the tissue act as stimulus for the secretion of lipoprotein into the blood stream and development of hyperlipidemia (Hulley and Gorden, 1981).

Several recent papers have suggested that high density lipoprotein, rather than low density lipoprotein, serve as the major source of cholesterol for instance, Gwynne *et al*. (1976) have incubated adrenal glands were three times greater than from LDL. Circulating serum lipoproteins provides cholesterol substrate for steroid genesis in the adrenal and ovaries of most species including man and the rat (Gwynne and Strauss, 1982; Strauss *et al*., 1981 and Brown *et al*., 1979). In the fasting state, two major lipoprotein classes, high density (HDL) and low density (LDL) lipoproteins, carry greater than 90% of circulating cholesterol. The relative amount of HDL predominant in rat (Mahley and Holcombe, 1977) and LDL predominant in man (Havel *et al*., 1955). The biological activity of lipoprotein particles is determined by their constituent apoproteins and lipids rather than by their density (Osberne and Brewer, 1977; Jackson *et al*., 1957). Considerable evidence now indicates that the testis (Schreiber *et al*., 1982) posses a distinct mechanism for accumulating HDL cholesterol.

The focus of the present investigation was to determine whether the blood glucose and ley dig cells of the testis are inter related with lipid and lipoprotein. In the testis, the capillary wall constitutes the first barrier for the transfer of plasma lipoproteins to the interstitium and Ley dig cells. Consumption of Sildenafil citrate and ethanol beverages is most common in human societies. The aim of the present study was to evaluate the Sildenafil citrate plus ethanol administration on lipids and lipoproteins in the testis tissue of Albino rats.

MATERIALS AND METHODS

Chemicals: Cholesterol, Chromotropic acid, glycerol trioleate and sodium periodate were purchased from Sigma chemical company, St. Louis, MO, USA. Sildenafil citrate (Viagra) was purchased from Sigma Aldrich, Inc. (USA). Ferric chloride, albumin and sodium arsenate were purchased from Ranbaxy (P) Ltd., New Delhi, India. Ethanol was obtained from Nellikuppam, Cuddalore District, South India. All other chemicals used were of analytical grade and were obtained from Central Drug House, New Delhi, India.

Experimental animals: Forty eight male Albino rats weighing 180-200 g were procured from the Central Animal House, Rajah Muthiah Medical College, Annamalai University. They were housed three per cage in plastic cages (47×34×18 cm), lined with husk, renewed every 24 h and had free access to drinking water and food. The animals were kept at room temperature (30±2°C) under semi natural light-dark conditions (12 h light/dark). The animals used in the present study were cared in accordance with the Ethical Committee for Animal Care of Annamalai University and the Indian National Law on Animal Care and use. (Register Number:166/1999/ CPCESA) (National Institute, 1985).

Study design: The animals were divided into eight groups and treated as follows.

Animals continued to receive standard pellet diet and isocaloric glucose from a 40% glucose solution daily by intragastric intubation and served as control group. Animals continued to receive standard pellet diet, Group 2 to Group 5 Short-term process for Sildenafil citrate (1 μg g^{-1} body weight) plus 18% ethanol (5 g kg^{-1} body weight) (Enomoto *et al*., 1999) administered for 1, 2½, 4 and 24 h. Group 6 to Group 8 long-term process for Sildenafil citrate plus ethanol every day by intragastric intubations for 15, 30 and 45 days.

The animals were monitored closely and average food in take was recorded every day in long-term animals. They were weighed both at the start and end of the experiment. The total experimental duration was 45 days. The animals were fasted overnight anaesthetized with an intra muscular injection of ketamine hydrochloride (30 mg kg^{-1} body weight) and sacrificed by cervical dislocation at the end of work. Blood collected from the carotid artery was allowed to coagulate at ambient temperature for 30 min. Plasma was separated by centrifugation at 2000 rpm. The lipids were extracted by the method of Folch *et al*. (1957). Total cholesterol was estimated by the method of Sackett (1925). Serum triglycerides were estimated by the method of Foster and Dunn (1973).

High-density lipoprotein (HDL) was estimated in the supernatant after precipitating the serum. Very low density lipoprotein (VLDL) content was calculated as follows

VLDL = Triglycerides/5
LDL = Total cholesterol-(HDL+VLDL)

Statistical analysis: All the results obtained are expressed as means±SD of six rat in each group. Statistical evaluation was done using analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The statistical significance was at a $p<0.05$ (Duncan, 1957).

RESULTS

Table 1 shows the average weight gained by the rats during the period of 30 days. But, there was no significant in body weight of the rats during the period of 45 days.

Table 1: Average weight gain by the animals (long-term) during the experimental

	Total body weight (g)			
Groups	Initial	15 days	30 days	45 days
Control	185.83±5.84	215.83±3.76	239.17±3.76	281.67±6.83
Drug+Ethanol	183.16±3.76	218.33±5.16	255.83±3.76	280.33±3.76
F-ratio	1.38	0.92	58.82	0.068

Values are means±SD of six rats from each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT), $p<0.05$ (ANOVA)

Table 2: Short-term effect of a single dose of Sildenafil citrate and ethanol on plasma lipid and lipoproteins of control and experimental animals

Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Control	67.45 ± 1.20^{a}	50.92 ± 1.19^{a}	$45.27\pm1.49^{a,b}$	$11.99\pm1.12^{b,c}$	10.18 ± 0.24^{a}
1 h	66.91 ± 1.31^{a}	$52.24\pm1.13^{a,b}$	45.83 ± 1.13^{b}	$10.63\pm1.71^{a,b}$	$10.45\pm0.23^{a,b}$
2½ h	66.96 ± 1.30^{a}	54.24 ± 1.08^{c}	46.50 ± 1.56^{b}	9.61 ± 0.93^{a}	10.85 ± 0.22^{c}
4 h	67.66 ± 1.24^{a}	$53.06\pm1.09^{b,c}$	43.85 ± 1.09^{a}	13.19 ± 0.28^{c}	$10.61\pm0.22^{b,c}$
24 h	67.43 ± 1.54^{a}	53.82 ± 1.24^{c}	43.81 ± 1.10^{a}	12.86 ± 1.76^{c}	10.76 ± 0.25^{c}
F-ratio	0.37	7.82	5.17	8.37	7.98

Values are means±SD of six rats in each group. Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Table 3: Long-term effect of continuous dose of Sildenafil citrate and ethanol on plasma lipid and lipoproteins of control and experimental animals

Groups	Cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})
Control	67.45 ± 1.20^{a}	50.92 ± 1.19^{a}	45.27 ± 1.49^{c}	11.99 ± 1.12^{a}	10.18 ± 0.24^{a}
15 D	81.40 ± 1.64^{b}	85.17 ± 1.64^{b}	$44.49\pm0.79^{b,c}$	19.77 ± 1.84^{b}	17.03 ± 0.33^{b}
30 D	103.32 ± 2.97^{c}	94.32 ± 1.68^{c}	$43.28\pm1.17^{a,b}$	41.18 ± 1.91^{c}	18.86 ± 0.34^{c}
45 D	126.20 ± 2.18^{d}	107.36 ± 2.10^{d}	42.93 ± 0.89^{a}	61.81 ± 1.11^{d}	21.47 ± 0.42^{d}
F-ratio	895.13	1230.13	5.61	1276.16	1237.89

Values are means±SD of six rats in each group.Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Table 4: Short-term effect of Sildenafil citrate and ethanol on testis tissue lipids of control and experimental animals

Groups	Cholesterol (mg g^{-1} tissue)	Triglycerides (mg g^{-1} tissue)
Control	4.39 ± 0.07^{b}	4.75 ± 0.08^{b}
1 h	4.24 ± 0.09^{a}	4.64 ± 0.09^{a}
2½ h	$4.30\pm0.07^{a,b}$	4.60 ± 0.07^{a}
4 h	4.54 ± 0.10^{c}	4.60 ± 0.07^{a}
24 h	4.54 ± 0.09^{c}	4.59 ± 0.07^{a}
F-ratio	15.40	4.18

Values are means±SD of six rats in each group.Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Table5: Long-term effect of Sildenafil citrate and ethanol on testis tissue lipids of control and experimental animals

Groups	Cholesterol (mg g^{-1} tissue)	Triglycerides (mg g^{-1} tissue)
Control	4.40 ± 0.07^{a}	4.76 ± 0.08^{a}
15 days	5.46 ± 0.12^{b}	5.94 ± 0.12^{b}
30 days	6.39 ± 0.13^{c}	6.86 ± 0.11^{c}
45 days	7.16 ± 0.10^{d}	7.42 ± 0.12^{d}
F-ratio	755.56	689.84

Values are means±SD of six rats in each group.Values not sharing a common superscript letter differ significantly at $p<0.05$ (DMRT)

Serum lipids: The serum lipids of all the 8 groups of animals were shown in Table 2 and 3. In short-term treatment groups cholesterol concentration was found to be any significant change as compared with those of the control rats. Whereas, in long-term treatment groups cholesterol levels were significantly high between Sildenafil plus ethanol treated and controls. In short - term treatment groups triglycerides levels were found to be significantly with minimum variation. Whereas, in long-term treatment groups triglycerides levels were significantly higher than control.

Testis lipids: Table 4 and 5 shows the concentrations of cholesterol and triglycerides in the testis of control and experimental rats. Cholesterol levels of the testis during short-term treatment period showed minimum activity. Whereas, in long-term treatment period showed higheractivity than control. Triglycerides levels of the testis during short-term treatment period showed decreased activity. Whereas, in long-term treatment period showed higher activity significantly than control.

Serum lipoproteins: Table 2 and 3 shows the concentrations of lipoproteins in the plasma of control and experimental animals. In short - term treatment period concentration of Low-density Lipoprotein (LDL) and Very-low Density Lipoprotein (VLDL) of plasma were significantly minimum than control. High-density Lipoprotein (HDL) concentration was no significantly variationthan control. Whereas, in long-term period treatment showed of plasma were significantly decreased in treated groups as compared with the control group, respectively.

DISCUSSION

The use of Sildenafil citrate for the treatment of erectile dysfunction by many patients. The cardiovascular disease has resulted due to properties of the drug (Gillies *et al*., 2002). Ethanol abuse in major causes of health problem and a public health issue (Choi *et al*., 1998). Ethanol is rich in calories and devoid of nutrients, thus contributing to accumulation of fat in the testis. On the other hand, ethanol is known to reduce the absorption of other foodstuff and nutrients from intestine (Mendenhall *et al*., 1969). Hyperlipidemia associated with ethanol consumption is relevant to the problem of atherosclerosis and heart disease in the drinking population. Elevated serum triacylglycerols, low-density lipoprotein cholesterol and decreased levels of HDL were shown to be risk factors for cardiovascular disease (Manninen *et al*.,1988). Ethanol caused a significant change in the metabolism of lipids and lipoproteins (Senthilkumar and Nalini, 2004). Serum and tissue cholesterol levels increase with ethanol consumption (Senthilkumar *et al*., 2002). Similar results have been correlated with the above finding.

Lipoproteins are chemically modified by oxidation. These oxidized or modified lipoproteins do not reach with LDL receptors, leading to esterification of cholesterol and conversion of macrophages to foam cells, there by contribution to the hyperlipidemia observed on ethanol consumption (Wetzles *et al*., 1993). HDL helps in scavenging cholesterol from the tissue in the presence of Lecithin Cholesterol Acyl Transferase (LCAT) and brings it to the tissue. In the present study, it has been observed that the HDL concentration in serum was significantly lower in rats received Sildenafil and ethanol than in control rats. Further more, an inverse association between moderate ethanol consumption and Coronary Heart Disease (CHD) has been observed in several epide miological studies (Ajani *et al*., 2000). This reduction in risk of Coronary Heart Disease (CHD) could be a consequence of a decrease in circulating levels of LDL cholesterol (Clevidence *et al*., 1995). However another important change in serum lipids in moderate drinkers is an increase in HDL. At least half of the reduced risk in cardiovascular disease associated with moderate ethanol consumption was attributed to change in circulating levels of HDL and HDL subtractions (Langer *et al*., 1992). These reports suggest that ethanol taken in moderation may prevent atherosclerosis.

Ethanol consumption leads to increased concentrations of plasma VLDL and LDL, as the release of these lipoproteins from the tissue apparently does not keep place with the rate of formation of triglycerides and the triglycerides accumulates in the tissue (David and Lawrence, 1988). Plasma LPL is an important enzyme responsible for the hydrolysis of triglycerides present in chylomicrons and VLDL (Parthasarathy *et al*., 1989). In the present study it is induct that the LDL and VLDL concentrations in serum were significantly higher in rats receiving Sildenafil and ethanol than in control rats.

It may be informed from the present study that Sildenafil citrate and ethanol consumption appeared to contribute to enhance higher cholesterol, higher triglycerides, lower HDL, higher LDL and higher VLDL, all of which may attribute increased risk of cardiovascular diseases (Seo *et al*., 2004). These results suggest that some of the effects of Sildenafil citrate and ethanol in takes caused similar disease among treated rats than control rats.

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