FTIR STUDIES ON THE CONCOMITANT EFFECTS OF SILDENAFIL CITRATE AND ETHANOL ON KIDNEY OF ALBINO MICE

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*Abstract***.** Male Wistar albino mice were co-administered with appropriate dose of sildenafil citrate and ethanol. The kidney samples of those animals were analysed for their lipid content using FTIR spectral technique. Specific extinction coefficient (*K*) values for various functional groups of lipids present in kidney samples of each group were determined. From the present investigation an enhanced level of lipid peroxidation was detected for 30 days animals treated concomitantly with sildenafil citrate and ethanol.

Key words: Albino mice, kidney, sildenafil citrate, ethanol, lipids, FTIR spectroscopy.

INTRODUCTION

The issue of erectile dysfunction (ED) provides a paradoxical situation to both patients and physicians. Sildenafil citrate is reportedly an effective and safe medication indicated for the treatment of ED. It is a competitive inhibitor of cGMP-specific phosphodiesterase type 5. The medication amplifies the effects of sexual stimulation by regarding the degradation of this enzyme. Sildenafil has been found effective in several subpopulations of men with ED, including sufferers from diabetes [1], hypertension, spinal cord injuries [7], multiple sclerosis [12], depression [10, 24] PTSD [18], men after resection of the prostate or radical prostatectomy [17], after renal transplant [22], men on dialysis [6] and men aged 65 years and older [4].

Studies conducted by Jeffcoate *et al*. [13] and Mc Cambridge *et al*. [3] show that men with ED are frequently chronic alcohol addicts. The findings of previous studies show that modest ethanol doses (e.g., at blood concentrations of $\leq$ 100mg/dL) can both increase sexual drive and decrease erectile capacity in men

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[5]. As a result, alcohol-dependent men commonly suffer from ED. Being an effective vaso-active agent currently available for the treatment of ED, most of them use sildenafil citrate. The combined use of sildenafil and alcohol may affect the biochemical balance of the body. Being the most sensitive organ, here we select kidney as the experimental organ.

Fourier transform infrared (FTIR) spectroscopy is more sensitive, rapid and more environment-friendly, compared to chemical methods. It is also a noninvasive technique that gives not only qualitative and quantitative information about molecular conformation, but also the interaction between neighbouring molecules. Thus, the technique detects macromolecular compounds such as proteins, lipids, carbohydrates and nucleic acids, simultaneously [27].

In this paper we present the results of our study to see whether the combined consumption of sildenafil citrate and ethanol affects the lipid concentration in the kidney tissues of albino mice.

MATERIALS AND METHODS

Drug, here, refers to the commercially available 50 mg tablet of sildenafil citrate (VIAGRA). Alcohol was purchased from sigma chemical Co. (St Louis, MO, USA). All other chemicals utilized were of analytical grade and were obtained from local firms (India).

Healthy male Wistar albino mice (*Mus musculus*), with an initial body weight of 25 – 30 grams, were used in this study. The animals were housed in stainless steel mesh cages, housed under controlled conditions (temperature 25±2 ºC, natural high-dark cycle). Commercial standard pellet diet (Hindustan lever Ltd, Mumbai, India) and drinking water were provided *ab libitum*. Six animals were usually tested as a group in each experiment. The commercial mice feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fiber (w/w), with adequate minerals and vitamin contents.

The animals were randomly divided into seven groups of six animals each.

- Group S1: Control animals treated intragastrically with conductivity water (1 lig/g body wt/day) for 30 days.
- Group S2: Animals received drug orally (at a rate of 1 µg/g body wt/day) for 15 days using intragastric tube
- Group S3: Animals received drug (at a rate of 1 µg/g body wt/day administered orally) for 30 days.
- Group S4: Animals were treated with alcohol orally (at a rate of 0.01 µg/g body wt/day) for 15 days using intragastric tube.
- Group S5: Animals were treated with alcohol orally (@ 0.01 µg/g body wt/day) for 30 days.
- Group S6: Animals received drug (@ 1µg / g body wt/day) followed by oral administration of alcohol. (@ 0.01 µg/ g body wt/day) for 15 days.

- Group S7: Animals received drug (@ 1 µg/ g body wt/day) followed by oral administration of alcohol (@ 0.01 µg/ g body wt./day) for 30 days.

After four hours of drug administration, the animals were sacrificed by cervical decapitation. The kidney was dissected out and quickly rinsed in 4% saline.

The kidney samples were collected from the control and the experimental albino mice. These samples were first dried to remove the moisture content. Then, the dried sample was finely ground by using Agate mortar. A known amount of this finely ground solid sample was intimately mixed with spectroscopically pure and dry potassium bromide (KBr) in the ratio 1:100. KBr pellets with 13 mm diameter and about 1 mm thickness were prepared and analysed using NICOLET AVATAR-360 FTIR spectrometer available at CISL, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

In the experiment we use NICOLET AVATAR-360 FTIR spectrometer. It consists of a Michelson's interferometer with frictionless electromagnetic drive and digital dynamic alignment. Helium Neon laser was used as reference laser. High performance D-TGS detector was used for detection. Used spectral range was 7400–375 cm^{-1}. Peak to peak noise is less than 2.2×10^{-5} Abs.

RESULTS AND DISCUSSION

Albino mice, the animals chosen for the present study, were administered with sildenafil citrate (Caverta) and alcohol, and the kidney samples were subjected to FTIR spectral analysis.

Figs. 1 to 7 illustrate the FTIR spectra of kidney sample of control and drug treated experimental animals. Prominent bands appear in the spectra at 3276 cm^{-1}, 2954 cm^{-1}, 2923 cm^{-1}, 2854 cm^{-1}, 1743 cm^{-1}, 1650 cm^{-1}, 1543 cm^{-1}, 1459 cm^{-1}, 1400 cm^{-1}, 1233 cm^{-1}, 1168 cm^{-1} and 1065 cm^{-1}.

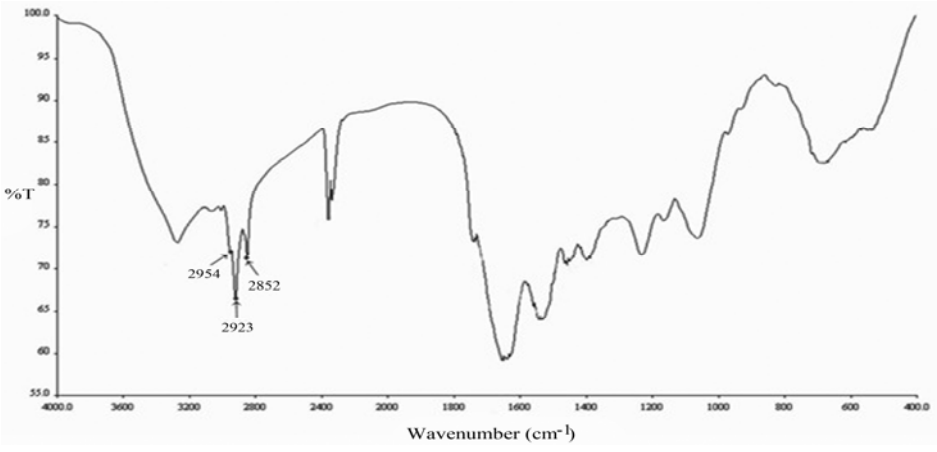

Fig. 1. FTIR spectrum of kidney of control (0 hr) albino mice.

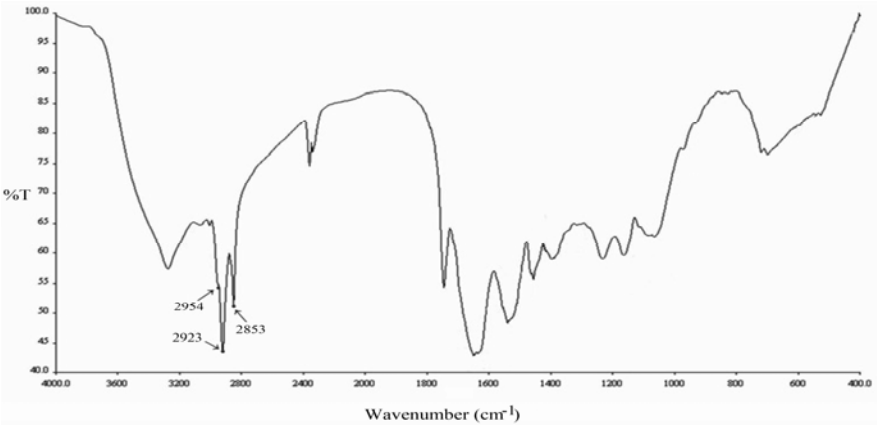

Fig. 2. FTIR spectrum of kidney of sildenafil citrate (15 days) treated albino mice.

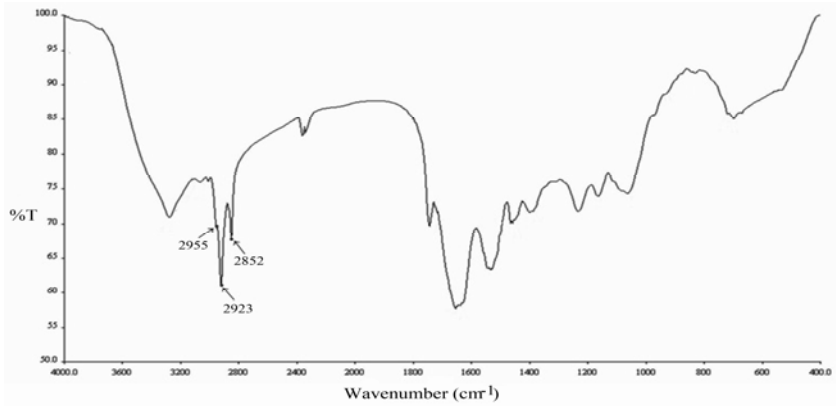

Fig. 3. FTIR spectrum of kidney of sildenafil citrate (30 days) treated albino mice.

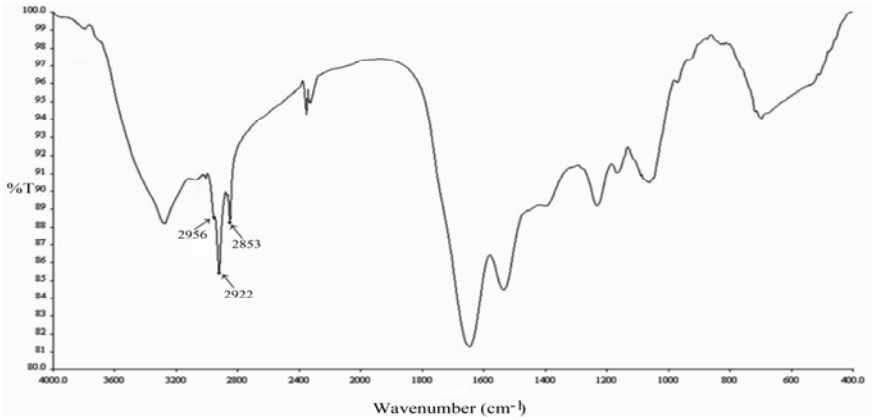

Fig. 4. FTIR spectrum of kidney of ethanol (15 days) treated albino mice.

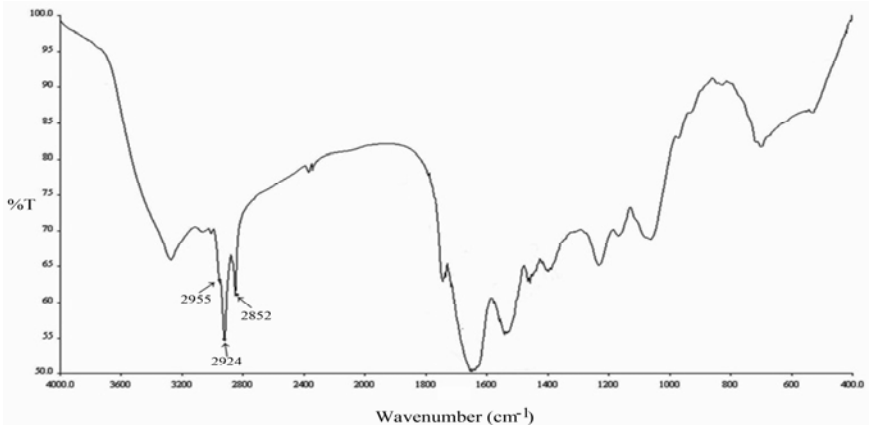

Fig. 5. FTIR spectrum of kidney of ethanol (30 days) treated albino mice.

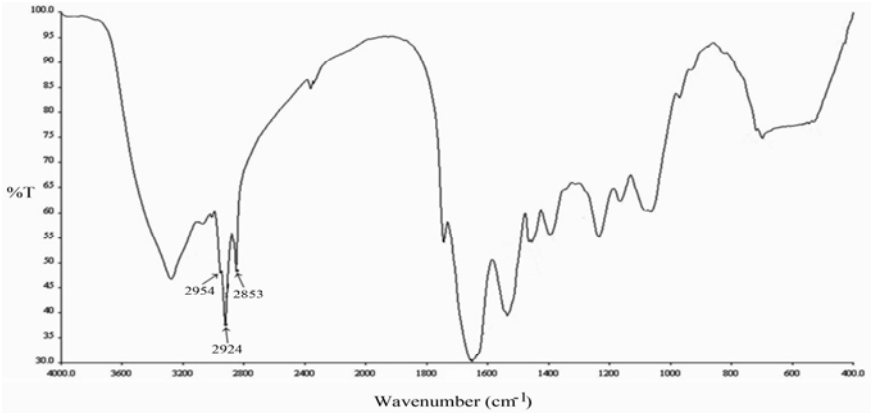

Fig. 6. FTIR spectrum of kidney of sildenafil citrate and ethanol (15 days) treated albino mice.

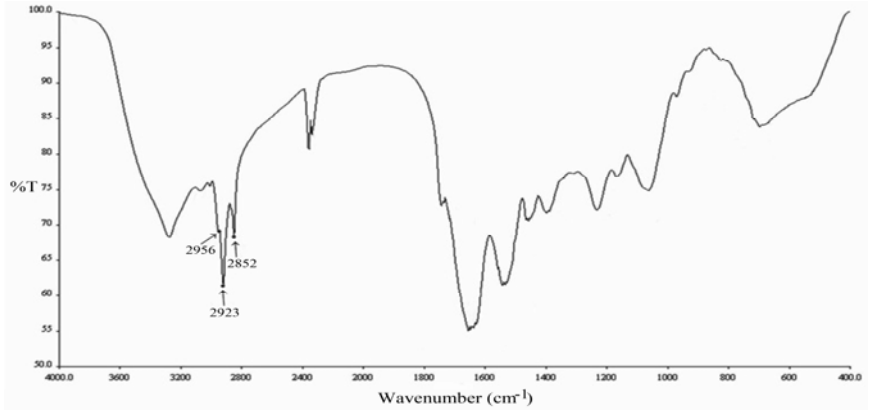

Fig. 7. FTIR spectrum of kidney of sildenafil citrate and ethanol (30 days) treated albino mice.

The FTIR spectra of kidney tissues are quite complex. There are several bands which appear in the 3000 – 2800 cm^{-1} and 1800 – 1000 cm^{-1} regions. Some of these bands, such as the Amide I band which appears at around 1650 cm^{-1}, need special care for data analysis since it consists of several unresolved bands. For this reason, in the present study, we only report the results of the C–H stretching region in which the main bands are: the CH_3 asymmetric stretching (2954 cm^{-1}), CH_2 asymmetric stretching (2923 cm^{-1}), CH_3 symmetric stretching (2872 cm^{-1}), CH_2 symmetric stretching (2852 cm^{-1}), CH_2 scissoring (1466 cm^{-1}) and the CH_3 bending (1388 cm^{-1}) vibrations. Table 1 shows the tentative assignment for prominent absorption bands of characteristic chromophoric groups of lipids.

Table 1

CH band assignments of major absorptions in IR spectra of kidney of experimental albino mice [2, 26]

Wave number (cm^{-1})	Probable chromophoric groups
2954	CH_3 (Asymmetric Stretching) vibrations mainly due to lipids
2923	CH_2 (Antisymmetric Stretching) vibrations mainly due to lipids
2872	CH_3 (Symmetric Stretching) vibrations mainly due to proteins
2852	CH_2 (Symmetric Stretching) vibrations mainly due to lipids
1466	CH_2 (Scissoring) vibrations mainly due to lipids
1388	CH_3 ((Bending) vibrations due to lipids

Prominent bands occurring at 2954 cm^{-1}, 2923 cm^{-1} and 2852 cm^{-1}, characteristic of the lipid bands, were chosen and the specific extinction coefficient (K) was calculated for all drugs treated and control groups by using the formula

$$K = DA/m \ (cm^2/g) \tag{1}$$

where D is the optical density of absorption band, A is the area of the pellet (in cm^2) and m is the concentration of the sample in the pellet in (g). These values are shown in Table 2.

Figs. 8 to Fig. 10 show the variation of specific extinction coefficient (K) for absorption bands at 2954 cm^{-1}, 2923 cm^{-1} and 2852 cm^{-1} of characteristic chromophoric groups of lipids.

The results of the present FTIR investigation can be discussed as follows:

Infrared band occurring at 2954 cm^{-1} represents the CH_3 asymmetric stretching vibrations of the chromophoric groups of lipids. The results of the present study show a prominent accumulation of lipids for S_3 (30 days drug treated), S_5 (30 days ethanol treated), S_6 (15 days drug and ethanol-treated) and S_7 (30 days drug and ethanol treated) groups of animals. However, extinction coefficient (K) indicates the high concentration of the chromophoric heavy loading of lipid has been noticed only for S_3 (30 days drug treated) and S_7 (30 days drug and ethanol treated) groups of albino mice (Table 1, Fig. 8).

Table 2

Semi-quantitative FTIR determination of lipids in kidney of experimental albino mice

Band (cm^{-1})	Sample	Specific extinction coefficient (K) (cm^2/g)
2954	S_1	88.39
	S_2	128.88
	S_3	248.36
	S_4	43.02
	S_5	159.14
	S_6	130.65
	S_7	234.60
2923	S_1	119.86
	S_2	172.28
	S_3	352.37
	S_4	55.16
	S_5	210.18
	S_6	171.16
	S_7	319.41
2852	S_1	93.93
	S_2	136.63
	S_3	276.15
	S_4	43.02
	S_5	170.51
	S_6	133.21
	S_7	241.45

S_1 – Control, S_2 – Drug (15 days), S_3 – Drug (30 days), S_4 – Alcohol (15 days)
S_5 – Alcohol (30 days), S_6 – Drug + Alcohol (15 days), S_7 – Drug + Alcohol (30 days)

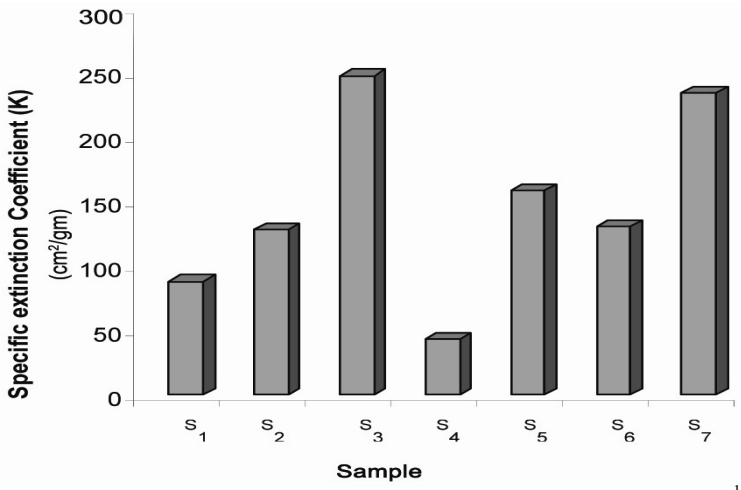

Fig. 8. Changes in specific extinction coefficient (K) for IR band at 2954cm^{-1} for kidney of experimental albino mice.

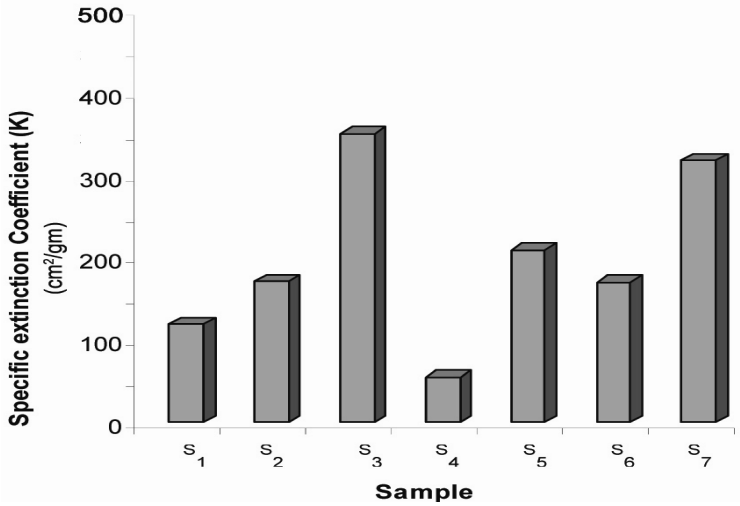

Fig. 9. Changes in specific extinction coefficient (K) for IR band at 2923cm^{-1} for kidney of experimental albino mice.

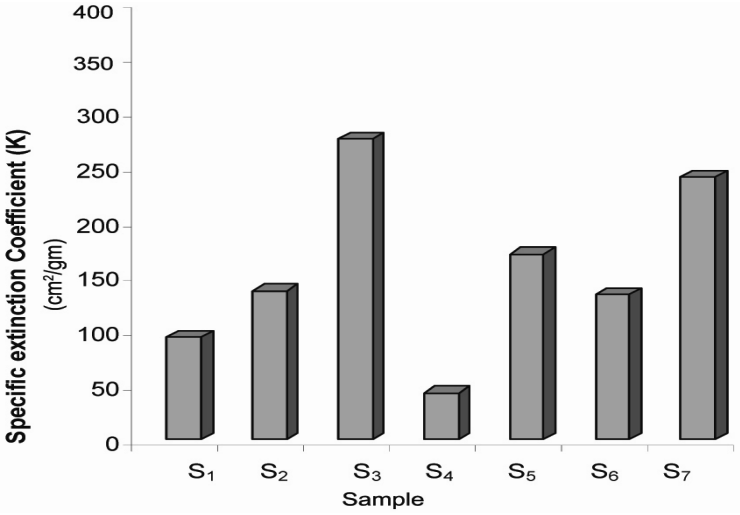

Fig. 10. Changes in specific extinction coefficient (K) for IR band at 2852cm^{-1} for kidney of experimental albino mice.

Absorption frequency at 2923 cm^{-1} denotes the antisymmetric stretching vibrations of CH_2 groups representing mostly of lipids. The trend of lipid accumulation in kidney of albino mice, as detected based on 2923 cm^{-1} band has been found to be similar to that of the absorption frequency at 2954 cm^{-1}. The level of lipid concentration in kidney has been observed to increase with the duration of the drug treatment and found to be the highest for S_3 (30 days drug treated) group.

Noticeable changes in the concentrations of lipid have been detected for S_5 (30 days ethanol treated), S_6 (15 days drug and ethanol treated) and S_7 (30 days drug and ethanol treated) groups of albino mice (Table 1, Fig. 9).

Infrared absorption band occurring at 2852 cm^{-1}, representing the CH_2 (symmetric stretching) vibrations mostly of the chromophoric groups of lipids, has also been observed to exhibit a similar trend in lipid accumulation, as that of 2954 cm^{-1} and 2923 cm^{-1} bands. A striking change in the lipid level has been detected for S_3 (30 days drug treated), S_5 (30 days ethanol treated), S_6 (15 days drug and ethanol treated) and S_7 (30 days drug and ethanol treated) groups, as compared to S_1 (control) group of animals (Table 2, Fig. 10).

In 3000–2800 cm^{-1} spectral region lipids are the major contributors [16]. Hence, high concentration of absorption bands indicates high concentration of lipid [20]. In our study, it is revealed that heavy loading of lipids occurs in the kidney tissues of sildenafil and ethanol-treated albino mice. Hence, we can say that the co-administration of sildenafil and ethanol-enhances the lipid accumulation in kidney tissues. In other words, sildenafil and ethanol, both have a tendency to increase the level of cholesterol in kidney tissues.

Similar results indicating an accumulation of lipid in the heart and liver tissues have been observed by Feride Severcan [11] for STZ – induced albino rats.

From the present study, it is clear that the co-administration of sildenafil citrate and alcohol enhances lipid accumulation in kidney tissues of experimental albino mice. The lipid accumulation in the rat kidney during lead intoxication has been reported by Rajagopalan Sivaprasad *et al*. [21]. According to Osbaldo Ramos *et al*. [19], arsenic intoxication has also been noticed to increase lipid accumulation in rat tissues.

Another study conducted by Zhi Zhong Guan *et al*. [29] revealed that higher doses of fluoride also alter the lipid composition and lipid peroxidation in kidney tissues of rats. The disorders in lipid content of biological tissues resulted in alterations in the activity of a number of biologically active enzymes [8].

A study conducted by Engelbardt [9] indicates a relationship between the lipid accumulation and lipid peroxidation. Therefore, we can say that the combined dosage of sildenafil citrate (Caverta) and ethanol may enhance the level of lipid peroxidation. These results were confirmed with our own UV-VIS study, where we have also observed alterations in Antioxidants level in drug treated tissues. In that particular study, we noticed decreased activities of antioxidant enzymes like SOD, GSH, GPx and CAT which indicate the failure of antioxidant defence system [23]. Thus the inhibition of enzymes involved in free radical removal leads to the accumulation of H_2O_2, which promotes lipid peroxidation and modulation of DNA altered gene expression and cell death [24, 28].

CONCLUSIONS

In conclusion, co-administration of sildenafil citrate (Caverta) and ethanol enhances lipid accumulation in kidney tissues of experimental albino mice. Lipid accumulation in Kidney adversely affects the biological functions of the kidney. Evidences show that lipid accumulation in biological tissues seriously affects the cell functions as well as many pathological processes [14]. Also, sildenafil citrate and alcohol disrupt the antioxidant balance of tissues that leads to biochemical and physiological dysfunction [23].

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