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β-sitosterol augment antihyperlipidemic effect in hyperlipidemic rats induced by Triton

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ABSTRACT

AIM**:** β-sitosterol augment antihyperlipidemic effect in hyperlipidemic rats induced by Triton**.** MATERIAL & METHODS**:** Leaves of *Corchorus trilocularis* were extracted by using chloroform and other solvents. Since Chloroform extract showed better activity as compared to other extract so it was selected for further isolation and characterization of active compound. The compound was isolated by using column chromatography and suitable solvent systems i.e. chloroform: methanol (74:26). β-sitosterol was isolated by using this solvent system. Its acute toxicity studies were performed for the dose determination and further antihyperlipidemic evaluation. RESULTS**:** IR (KBr) 3460, 2868, 1731, 1644, 1512, 1455, 1421, 1350, 1298, 1249, 1201, 1108, 950, 849, 726, 552cm^{-1} ^{1}H NMR (400 MHz, Chloroform) δ 5.48 (s, 5H), 3.42 (s, 5H), 2.25 (s, 4H), 2.02 (s, 4H), 1.88 (d, *J* = 18.0 Hz, 9H), 1.77 (s, 4H), 1.70 (d, *J* = 9.2 Hz, 11H), 1.66 – 1.56 (m, 15H), 1.48 (dd, *J* = 15.0, 7.2 Hz, 21H), 1.42 – 1.12 (m, 72H), 1.16 – 1.12 (m, 18H), 1.15 – 1.08 (m, 27H), 1.08 – 0.90 (m, 92H), 0.85 (s, 6H), 0.73 (s, 4H). ^{13}C NMR (100 MHz, Common NMR Solvents) δ 141.20 (s), 121.64 (s), 71.05 (s), 57.01 (s), 56.46 (s), 51.13 (s), 45.02 (s), 43.08 (d, *J* = 9.0 Hz), 39.72 (s), 37.77 (s), 37.16 (s), 36.72 (s), 35.41 (s), 32.66 (s), 32.31 (s), 31.60 (s), 31.13 (s), 28.32 (d, *J* = 4.1 Hz), 24.97 (d, *J* = 2.6 Hz), 21.27 (s), 19.99 – 19.67 (m), 18.84 (d, *J* = 13.2 Hz), 13.00 (s), 12.11 (s). β-sitosterol at the dose of 10 and 20 mg/kg showed highly significant (p<0.001) and moderately significant (p<0.01) effect on lipid profile in comparison to that of hyperlipidemic group. CONCLUSION: The noticed antihyperlipidemic impact might be because of improved glycemic control and expanded plasma insulin movement, which permits the rodents treated with β-sitosterol to keep up plasma and tissue lipids in ordinary level.

KEYWORD: *β-sitosterol, Triton Induced Hyperlipidemia, HDl, VLDL, Chloroform Extract*

PRESENTATION:

The World Health Organization (WHO), American Heart Associations, and disease transmission experts approximately the world has been articulating an alert on the quick expansion in the weight of heart illnesses for as long as Twenty years. Trial and epidemiological examinations have demonstrated that the plasma hyperlipidemic condition could add to the advancement of atherosclerosis and cardiovascular-framework associated illnesses [1]. Cardiovascular infections (CVDs) are the most well-known reason for horribleness and mortality in a large number of non-

industrial nations [2,3]. The legitimate procedure, to forestall probably to the delicacy atherosclerosis and diminish the frequency of cardiovascular illness occasions, is to objective hyperlipidemia either by medications or nutritional intercession [4, 5]. In light of this measure, various plants have gotten consideration and have been appeared to bring down plasma lipid levels [6]. Prior investigations likewise uncover that the utilization of such medicinal plants having polyphenols and glycoside-rich fractions diminish the danger of hyperlipidemia [7–10].

There are many traditionally reported medicinal plants that claim for the antihyperlipidemic activity and also claim their support in diabetic complications. Leaves of Corchorus trilocularis is such a well-documented plant which is traditionally reported for the treatment of hyperlipidemic condition. In our previous study, chloroform extract showed potent activity against hyperlipidemia so the current aim of present investigation is to isolate the active constituents from chloroform extract and its evaluation in hyperlipidemic rats.

MATERIAL & METHODS

MATERIALS

Equipments: The following equipments were used throughout the study.

Name of Equipment	**Model**
Digital Weighing Balance	Mettler
Digital pH meter	Equip-tronics
Incubator	Thermolab
Tissue Homogenizer	Remi-motors
Laboratory Centrifuge	Remi-motors
Electronic balance	Sartorius, CP2248
Hot air oven	Lab Tech
Water bath	Lab Tech
Soxhlet apparatus	ASGI
Rotatory Evaporator	Popular India
Melting Point apparatus	Lab. Hosp. Corporation
UV- Visible spectrophotometer	Shimadzu, Pharmaspec-1700
UV lamp	Camag
FTIR	Shimadzu 8400 S FT-IR
^{1}H- NMR	Brucker multinuclear FT NMR
^{13}C- NMR	Brucker
Mass spectrometer	TOF MS ES+ Mass spectrometer

Glass wares: Borosil and ASGI make glass wares were used.
Chemicals: All chemicals used were of analytical grade.
Drugs: Free sample of Triton was procured from Nicholas Piramal, INDIA.
Animals: Albino rats of Wistar species of either sex (150 to 200 g) were purchased, approved vendors for *in-vivo antihyperlipidemic* activity.

METHODS

Purchase and Identification of Leave Plant: The leaves of Corchorus trilocularis were unblown from the mountainous region of northern Iraq during September, showing a green color with a rough surface. The leaves of the plant were washed well in tap water, dried in the shade, then powdered well, and used in successive extraction methods. Plants were identified by Dr. Ghassan.

Successive extraction methods:

The correctly identified plant leaves are dried in shadow at room temperature & after 4-5 days, it is formed in powder by mixer grinder. Powdered drug 100gm was weighed and packed in soxhlet. The drug was continuously extracted with petroleum ether for about 72 hours. Complete defatting was ensured by placing a drop from the thimble on a filter paper to give an oily spot. The mare was dried in the air to remove traces of petroleum ether. The defatted drug was subjected to extraction with chloroform in soxhlet apparatus, the extraction was completed in 17-18 hrs. The extract was dried & stored in a dark place. Dried marc was subjected to extraction with ethanol (90%) and finally butanol in soxhlet apparatus, the extraction was completed in 25 cycles [8].

Phytochemical Test

Preliminary screening was performed meant for the existence of various active constituents i.e. alkaloids, flavonoids, terpenoids, steroids, etc. [9,10].

Experimental Animals

Wistar Albino rodents of one or the other sex (150 to 200 g) were bought by an endorsed merchant. They were kept up under research center conditions at 25 ± 2°C and an ordinary 12-hour light-dim cycle was utilized for the analysis. and water was given not indispensable over the span of study.

Bioactivity guided isolation from chloroform extract of *Corchorus trilocularis*

In our previous experiments, chloroform extract showed potent activity. So for further isolation, chloroform extract was selected. 20 gm of the extract was mixed with 50 gm of silica gel and a very small amount of an appropriate solvent. This mixture was triturated in a mortar till a homogenous and dry free-flowing mixture was obtained. For the isolation of compounds, chloroform and methanol was used as a solvent system. Firstly 100% chloroform was passed through column and then an increasing quantity of methanol was used in different ratios (90:10, 80:20, 70:30, 60:40, and finally 50:50). Elute was collected at the rate of 25 drops per minute and each fraction was about 25 ml and a total of 250 fractions was collected. All the collected fractions were monitored simultaneously on a TLC plate using chloroform: methanol (74:26) as solvent system. The fractions showing the same TLC pattern were mixed together to make 3 fractions (F1-F3). The percentage yield of collected elutes was determined in deference to the total weight of the fraction. All the fractions were evaluated for antihyperlipidemic activity (data not shown here) and fraction 2 showed a single spot in TLC and it was characterized by spectral techniques. Test for steroids: A few crystals of the isolated compound were dissolved in chloroform and 1-2 ml acetic anhydride was added with 2 drops of conc. H_2SO_4 from the side of the tube. Red color, then blue and finally green color appears [8].

Spectral analysis and Structure elucidation

It was tried to purify compounds, which were obtained by employing Column chromatography and TLC and by re-crystallizing them in different solvents. The compounds were weighted and their melting point determined. Tools most widely used for the structure elucidation of natural products are Fourier transform infrared spectroscopy (FTIR), mass spectroscopy (MS), and nuclear magnetic resonance (NMR). With these tools, the structures of most natural products can be determined.

Acute Toxicity Study

Miller and Tainter (graphical) method were used to calculate LD50 of isolated compound. Overnight fasted adult Wistar albino rats were divided into 5 groups; 10 rats (5 male and 5 female) in each group. 5 dose levels were selected such that one dose with no mortality and a higher dose with 100% mortality; other 3 doses were in between. Animals were noticed for an initial 2 h and afterward at sixth h for any poisonous manifestations. After 24 h the quantity of perished rodents was included in each gathering and % mortality was determined. For the appraisal of action 1/tenth of LD50 was chosen [11].

Preparation of Doses

Dosages equal to the unrefined medication body weight were determined, and prepared in Tween 80 in a concentration of 1% w/v for the trial.

WR-1399-(Triton) the generate by Hyperlipidemia:

Hyperlipidemia was prompted in exploratory rodents utilizing Triton WR 1339 by following the previous technique [12–14]. Fluid arrangement of Triton WR 1399 was made in typical saline and infused at a portion of 250mg/kg., i.p. The detached compound was managed in a portion of 10, 20 mg/kg given [15, 16]. Atorvastatin (ATV) tablet was utilized as a norm at a portion of 30 mg/kg. All the medications were prepared in 0.5% w/v sodium CMC and were arranged newly each time. After the acclimatization time frame, creatures were isolated into five gatherings of 6-rodents each: Group I: Normal, Group II: Triton acceptance, Group III: Triton enlistment in addition to ATV (30 mg/kg/day, for about fourteen days) pretreated gathering, Group IV, V: Triton acceptance and sitosterol (10mg/kg/day and 20 mg/kg/day for2weeks) pretreated gathering. After pretreatment with sitosterol and ATV for a time of about fourteen days, the creatures were kept in wire-stunned enclosures for the duration of the day to restrict coprophagia. They were famished for 24 hours previous to the examination however permitted free admittance to water [17]. Blood tests were gathered from for the time being abstained rodents after 1hr after the last test drug management to separate gatherings for 0th day. oward the finish of the study, all the rodents were anesthetized for the blood assortment. Blood was gathered through sino-orbital cut utilizing EDTA as anticoagulant and the plasma isolated was put away at - 80°C and utilized for the lipid and lipoprotein assessment.

Biochemical parameters assessment:

Full-scale cholesterol and greasy oils levels in plasma were performed with an enzymatic technique for Accurex unit, Accrex Biomedical Pvt. Ltd., using a semi-electronic biochemical analyzer (Star 21 Plus auto analyzer, Rapid Diagnostics). HDL-cholesterol obsessions were estimated by a comparable procedure as used to choose full-scale cholesterol after ejection of various lipoproteins by precipitation with HDL precipitant. The LDL and VLDL cholesterol was controlled by the Friedwald condition [18].

Data configuration:

All information is accounted for as a mean ± standard mistake mean (SEM). The factual investigation was finished utilizing Graph cushion crystal 4.0 for the windows bundle. Single direction examination of change (ANOVA) was performed trailed by posthoc Tukey's test.

RESULTS

Phytochemical Test

Preliminary phytochemical screening exhibited the occurrence of various active constituents i.e. alkaloids, flavonoids, terpenoids, steroids in chloroform extract, etc.

Acute Toxicity Study

β-sitosterol does not show some poisonous symptoms at a dose of 200 mg/kg. So as per OECD guidelines, 1/10th and 1/5th dose of β-sitosterol i.e. 10 and 20 mg/kg were used for the entire study.

Characterization and Identification of compound

The compound gave tests for steroids and alcohols given by β-sitosterol. The β-sitosterol is a white crystalline needle-like substance. The melting point of β-sitosterol was 133°C respectively. Mass spectrum of Compound I illustrate a parent molecular ion [M+H] + peak at m/z 414 respectively which corresponds to the molecular formula C29H50O. The IR, 1H-NMR, and 13C-NMR data of β-sitosterol are as follow:

β-sitosterol

IR (KBr) 3460, 2868, 1731, 1644, 1512, 1455, 1421, 1350, 1298, 1249, 1201, 1108, 950, 849, 726, 552cm^{-1}

^{1}H NMR (400 MHz, Chloroform) δ 5.48 (s, 5H), 3.42 (s, 5H), 2.25 (s, 4H), 2.02 (s, 4H), 1.88 (d, *J* = 18.0 Hz, 9H), 1.77 (s, 4H), 1.70 (d, *J* = 9.2 Hz, 11H), 1.66 – 1.56 (m, 15H), 1.48 (dd, *J* = 15.0, 7.2 Hz, 21H), 1.42 – 1.12 (m, 72H), 1.16 – 1.12 (m, 18H), 1.15 – 1.08 (m, 27H), 1.08 – 0.90 (m, 92H), 0.85 (s, 6H), 0.73 (s, 4H).

^{13}C NMR (100 MHz, Common NMR Solvents) δ 141.20 (s), 121.64 (s), 71.05 (s), 57.01 (s), 56.46 (s), 51.13 (s), 45.02 (s), 43.08 (d, *J* = 9.0 Hz), 39.72 (s), 37.77 (s), 37.16 (s),

36.72 (s), 35.41 (s), 32.66 (s), 32.31 (s), 31.60 (s), 31.13 (s), 28.32 (d, *J* = 4.1 Hz), 24.97 (d, *J* = 2.6 Hz), 21.27 (s), 19.99 – 19.67 (m), 18.84 (d, *J* = 13.2 Hz), 13.00 (s), 12.11 (s).

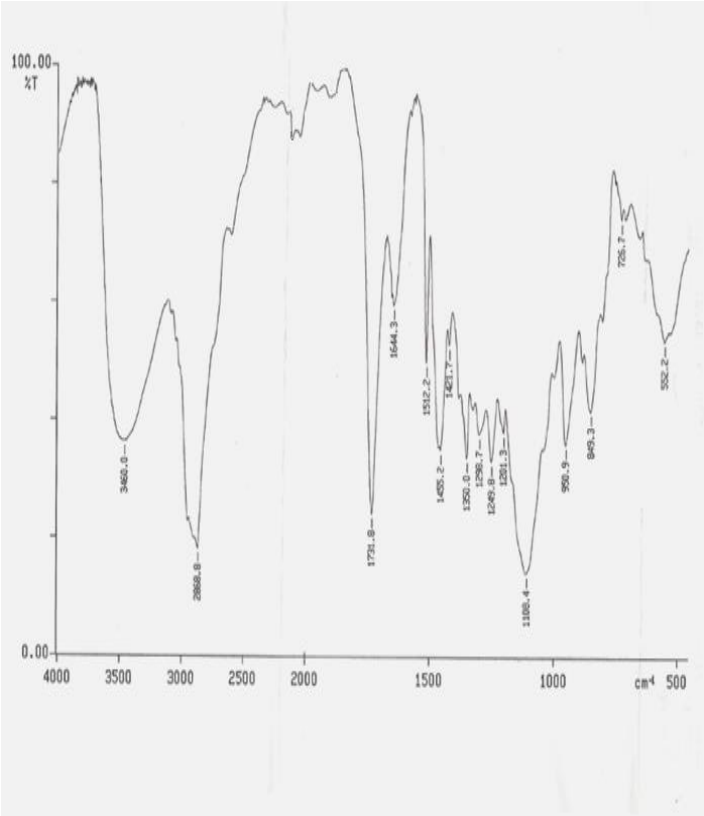

Fig. No. 1: IR Spectra of β-sitosterol

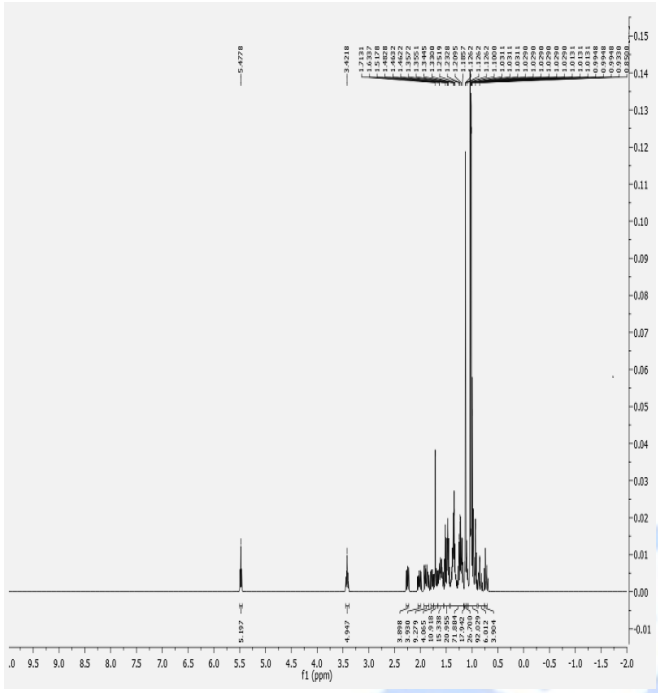

Fig. No. 2: ^{1}H-NMR Spectra of β-sitosterol

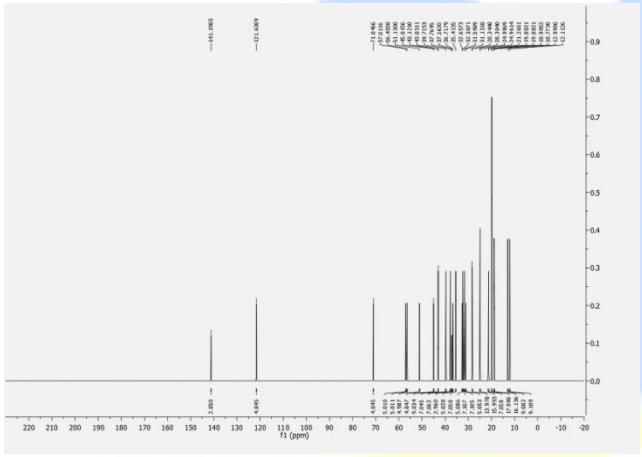

Fig. No. 3: ^{13}C-NMR Spectra of β-sitosterol

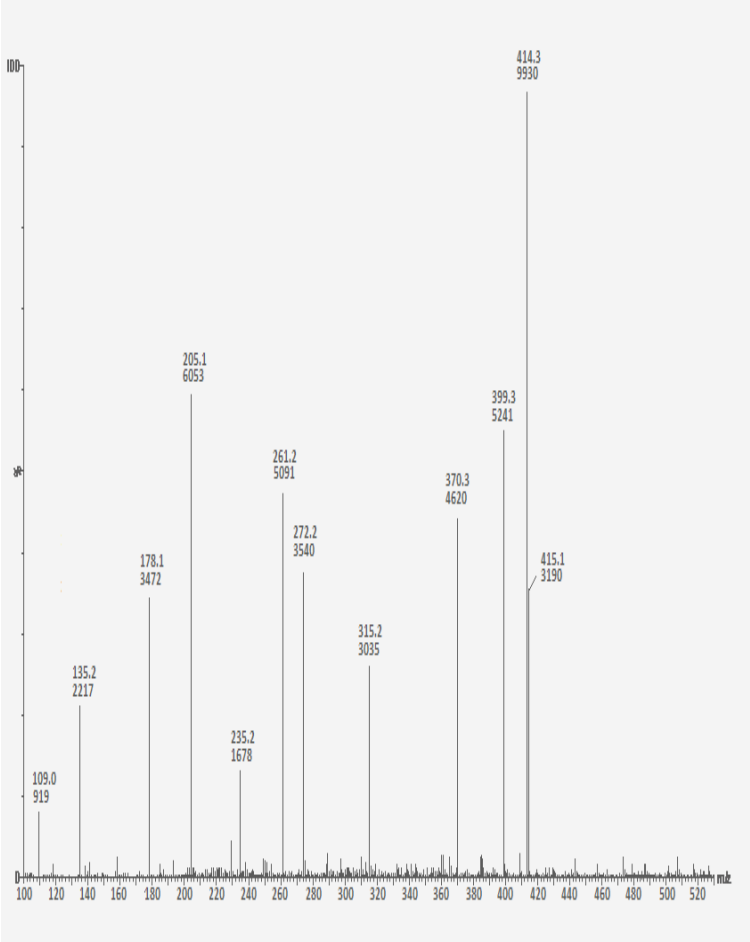

Fig. No.4 : Mass Spectra of β-sitosterol

Effect of β-sitosterol on different lipid level

Untreated rats showed significant hypercholesterolemia, hypertriglyceridemia, elevated LDL-Cholesterol, VLDL-Cholesterol, and decrease in HDL –Cholesterol in comparison to that of the normal group. β-sitosterol showed a very good effect on the lipid profile. β-sitosterol at the dose of 10 and 20 mg/kg showed a highly significant ($p<0.001$) and moderately significant ($p<0.01$) effect on lipid profile in comparison to that of the hyperlipidemic group. β-sitosterol also showed a highly significant effect on various lipids and also increased HDL level as compared to the disease groups or animals.

Table No.1: Effect of β-sitosterol on different lipid level in hyperlipidemic rats

S. No.	Groups	Parameters

		Total Cho-les-terol (mg/dl)	Triglycer-ides (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL-C (mg/dl)
1	Normal Control	78.17±2.33	77.12±2.43	16.59±2.16	16.26±1.34	50.49±2.39
2	Disease Control	107.44±2.66	106.39±1.31	55.67±1.97	23.17±1.17	33.18±1.18
3	β-sitosterol (10 mg/kg)	90.47±2.23**	92.48±2.41**	30.23±2.82***	20.32±2.16*	42.24±2.75**
4	β-sitosterol (20 mg/kg)	82.31±1.23***	86.31±2.59***	22.68±1.99***	15.29±2.93***	45.17±1.90***
5	Atarovastatin (250mg/kg)	81.23±2.19***	81.34±2.36***	19.33±1.66***	13.39±1.14***	46.74±3.29***

*Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups*

DISCUSSION

Drawn out the introduction to free extremists is a vital reason for tissue pressure and injury. The free extreme perpetual harm to tissue structures results from a lasting change in the sub-atomic example of sugars, lipids, proteins, and even nucleic corrosive bases [19].

The ascent in plasma triacylglycerols, cholesterol, and LDL-cholesterol levels in the current investigation demonstrates unsettling of lipid digestion and expanded occurrence of cardiovascular brokenness in diabetic rodents. Then again, glucagon and different hormones upgrade the breakdown of lipids. The stamped hyperlipidemia that describes the diabetic state may, hence, be viewed as a result of outgoing activities of lipolytic hormones on the fat stores [20]. Studies on hyperlipidemia in test creatures have recommended that an expansion in circulatory VLDL and their related fatty oils are to a great extent because of inadequate freedom of these particles from the flow [21].

Ordinarily coursing LDL-C goes through reuptake in the liver by means of explicit receptors and gets cleared from the dissemination [22]. HDL-C is defensive by turning around cholesterol transfer, restraining the oxidation of LDL-C, and killing the atherogenic impacts of oxidized LDL-C. The expanded degrees of LDL-C and VLDL-C decline HDL-C as there is a proportional connection between the centralization of VLDL-C and LDL-C. In hyperlipidemic rodents treated with β-sitosterol demonstrated a height in HDL-C and decrease in LDL-C and VLDL C. As there is a cozy connection between the absolute cholesterol level of raised plasma and the event of atherosclerosis, the capacity of β-sitosterol is reflected in the particular decrease of complete cholesterol through the decrease of VLDL and LDL segments. It very well may be valuable in forestalling atherosclerotic conditions, accordingly lessening the chance of coronary illness. It is consequently significant that the impact of β-sitosterol on plasma HDL, unmistakably shows that the degree of this lipoprotein portion expanded with the β-sitosterol organization. Cholesterol is an incredibly dangerous feature for some coronary heart sicknesses (CHD). The level of hypercholesterolemia is legitimately corresponding to seriousness in hyperlipidemia. In the current examination, we have noticed a more significant height of cholesterol in tissues of hyperlipidemic rodents. The expanded degree of cholesterol in

tissues could be because of the diminished degree of HDL-cholesterol. This, thus, brings about diminished expulsion of cholesterol from additional hepatic tissues by the HDL cholesterol [23,24]. Organization of β-sitosterol to hyperlipidemic rodents standardizes plasma levels of cholesterol because of the diminishing in cholesterol assimilation from the digestive tract, by official with bile acids in the digestive system and expanding bile acids discharge.

Amassing of fatty substances is one of the danger factors in CHD. Expanded fatty oil and diminished HDL cholesterol levels are the critical attributes of dyslipidemia in sort 2 diabetes [25]. Hyper triglyceridemia in sort 2 diabetes can result from an expanded hepatic VLDL over creation and hindered catabolism of fatty oil loaded particle. The capacity of lipoprotein lipase (LPL), a vital compound in the expulsion and corruption of fatty oils from dissemination, is constricted by in cooperation with insulin hardship and insulin obstruction. The brokenness of LPL adds to hypertriglyceridemia in the fasting and postprandial states. It has been hypothesized that a high plasma fatty oil level impacts LDL size and thickness from side to side a pattern of lipid trade [26].

Abundance of unsaturated fats in plasma delivered during diabetes advances changes of the overabundance of unsaturated fats into phospholipids and cholesterol in the liver. These two essences alongside abundance of fatty oils shaped simultaneously in the liver might be released into the blood as lipoproteins [27]. The expanded convergence of free unsaturated fats was seen in the liver and kidney of diabetic rodents and this might be because of lipid break and this may basis over the creation of NADPH, which brings about the initiation of NADPH subordinate microsomal lipid peroxidation. The organization of β-sitosterol diminished the free unsaturated fat in the tissues of hyperlipidemic rodents.

CONCLUSION

The degrees of all-out cholesterol (TC), fatty oils (TGs), low thickness and extremely low-thickness lipoproteins (LDL and VLDL) essentially expanded and HDL (plasma) was altogether diminished in hyperlipidemic rodents. Oral organization of β-sitosterol to hyperlipidemic rodents altogether switched these progressions towards close to ordinary. The noticed antihyperlipidemic impact might be because of improved glycemic control and expanded plasma insulin action, which permits the rodents treated with β-sitosterol to keep up plasma and tissue lipids at a typical level.

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