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IN-VIVO ANTIOXIDANT AND ANTIHYPERLIPIDEMIC ACTIVITY OF *SYZYGIUM CERASOIDEUM* EXTRACTS IN RATS

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ABSTRACT: Antioxidant and antihyperlipidemic activities of the extracts of *Syzygium cerasoideum* were investigated by studying their *in-vivo* effects on triton and cholesterol induced hyperlipidemia. Highest antioxidant activity was exhibited by methanolic extract followed by chloroform extract of *S. cerasoideum*. In triton induced hyperlipidemic model, the groups treated with the extracts of *S. cerasoideum* and pitavastatin demonstrated a significant decrease in the levels of TC, TG, LDL-C, VLDL-C particularly treatment with CESC at the dose of 200 mg/kg b.w resulted in significant decrease in levels of TC (67.60%), TG (47.89%), LDL-C (85.80%) and VLDL-C (47.89%) whereas HDL-C levels are significant to standard. In cholesterol induced hyperlipidemic model, the groups treated with the extracts of *S. cerasoideum* and pitavastatin demonstrated a significant decrease with the CESC and MESC at low doses in the serum levels TC (68.68%), TG (60.22%), LDL-C (85.44%), VLDL-C (70.71%) besides an increase in serum HDL-C (62.26%) levels when compared to cholesterol-induced hyperlipidemic control group. The treated groups of *S. cerasoideum* caused a significant decrease in the levels of SOD, CAT and GSH activities of MESC at the doses of 400 mg/kg/day and 10 mg/kg/day compared to the cholesterol-induced hyperlipidemic control group. It was observed from the histopathological findings that the rats fed with *S. cerasoideum* extracts showed a decrease in moderate microvesicular changes caused by cholesterol feedings. Results suggest that the methanolic followed by chloroform extracts of *S. cerasoideum* containing polyphenols and flavanols possess significant antioxidant and antihyperlipidemic activities.

INTRODUCTION: Experimental and epidemiological studies have shown that the plasma hypercholesterolemic state could contribute to the development of atherosclerosis and related cardiovascular system diseases, which are the most common cause of death [1].

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Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases, and cerebral vascular diseases [2]. Coronary heart disease, stroke, atherosclerosis, and hyperlipidemia are the primary cause of death [3].

It is characterized by elevated serum total cholesterol, low density, and very low-density lipoprotein cholesterol and decreased high-density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease [4]. Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with

the disease. The primary disease may be treated by using anti-lipidemic drugs, but the secondary originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treat of original disease rather than hyperlipidemia [5]. Hyperlipidemia is an metabolic disorder, specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein (VLDL-C) and triglycerides (TAG) with a concomitant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the circulation [6].

Medicinal plants play a significant role in the hypolipidemic activity; the literature suggests that the lipid-lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [7]. Many countries use medicinal plants as a folk medicine to treat hyperlipidemia and prevent atherosclerosis, a vast number of plants received attention in this regard and have been shown to lower plasma lipid levels [8].

Some of the plants reported for their hyperlipidemic activity mulberry [9], *Morus nigra* [10], *Cassia occidentalis* [11], *Dioscoreophyllum cumminsii* [13], *Hunteria umbellate* [12], *Curatella americana* [14], *Campomanesia adamantium* [15], *Labisia pumila* [16], *Morus* [17]. *Syzygium cerasoideum* plant belongs to the family Myrtaceae and native of India. Several active principles from this plant have been identified which include phenolic compounds cyanidin, 3-glucoside, delphinidin 3-glucoside, ellagic acid, kaempferol, myricetin, quercetin, quercetin and rutin [18]. The goal of this work is an evaluation of the antioxidant and antihyperlipidemic effect of *Syzygium cerasoideum* successive extracts in triton WR-1339 and high cholesterol diet-induced hyperlipidemia followed by *in-vivo* antioxidant activities respectively.

MATERIAL AND METHODS:

Preparation of *S. cerasoideum* Extracts: The plant was authenticated by Dr. Madhava Chetty, SVU Tirupathi was voucher specimen number 1320 was deposited for future reference and are prepared for successive extraction with pet-ether (40-60 °C), chloroform, ethyl acetate, and methanol. The extracts were prepared by continuous extraction using Soxhlet unit. The four extracts were then concentrated under reduced pressure to remove the solvent completely and lyophilized to be free from solvent and water [19].

Phytochemical Analysis: Phytochemical analysis was performed for all the extracts which include a test for steroids, triterpenoids, glycosides, flavanoids, saponins, alkaloids, tannins, carbohydrates, fat and oil according to the standard protocol [20].

High-Performance Thin-Layer Chromatography: Chromatography Conditions: High-Performance Thin-Layer Chromatography was performed on silica gel 60 F_{254} (10 cm × 10 cm; 0.25 mm layer thickness; Merck). SC extract and kaempferol (25 mcg/mL and 1 mcg/L prepared separately and collected and filtered through a 0.45-micron syringe filter 25 microliters of these extracts were subjected to HPTLC (CAMAG, Switzerland) analysis.

All these extracts and standard were spotted on a silica gel 60 $F_{25}4$ (Merck, Darmstadt, Germany) TLC plate. The plate was air dried and then developed by using the solvent system toluene: chloroform: methanol (7:2:1 v/v) as mobile phase in a CAMAG- twin-trough glass chamber previously saturated with mobile phase vapor for 20 min. After developing the plate, it was dried at 65 °C for 2 min, and then it was scanned using Scanner 3 (CAMAG, Switzerland) at 254 and 365 nm using WinCATS 4 software [21].

Animals Housing: Eighty-four Wistar rats (100-150g) were obtained from the Animal House, Dayananda Sagar University, Bengaluru. They were housed at a temperature of 24 ± 2 °C, 12 h light/ dark cycles, 35-60% humidity, in polypropylene cages and fed a standard rodent diet with water *ad libitum*. Animals were deprived of food but not water 4 h before the experiment.

Experimental procedures and protocols used in this study were approved by the Institutional Animal Ethics Committee of the DSU/Ph.D./IAEC/13/ 2017-18 CPCSEA conforms to the 'Guidelines for care and use of animals in scientific research' (Indian National Science Academy 1998, Revised 2000).

Anti-hyperlipidemic Activity of *S. cerasoideum*:

Triton WR-1339 Induced Hyperlipidemia: Triton WR-1339, a non-ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer), has been widely used to produce acute hyperlipidemia in animal models to screen natural or chemical drugs and to study cholesterol and triacylglycerol metabolism. The accumulation of plasma lipids by this detergent appears to be especially due to the inhibition of lipoprotein lipase activity. Triton WR-1339 (Sigma-Aldrich) was dissolved in normal saline (pH 7.4) and administered intraperitoneally to rats (400 mg/kg b.w) to develop acute hyperlipidemia in experimental animals [22].

Experimental Procedure: Animals were divided into 7 groups with 6 animals per group.

Group 1: Normal control.

Group 2: Hyperlipidemic control (20% w/v triton solution at pH 7.2 of 0.05M on 7th day).

Group 3: Hyperlipidemic treated with pitavastatin (10 mg/kg, p.o).

Group 4: Hyperlipidemic treated with chloroform extract (200 mg/kg, p.o).

Group 5: Hyperlipidemic treated with chloroform extract (400 mg/kg, p.o).

Group 6: Hyperlipidemic treated with the methanolic extract (200 mg/kg, p.o).

Group 7: Hyperlipidemic treated with the methanolic extract (400 mg/kg, p.o).

The animals were administered with corresponding treatments for 7 days. On day 7, 400 mg/kg triton WR-1339 was injected (i.p), to all the groups of rats immediately after drug administration. On the 7th day previous to drug treatment and after 24 h of triton administration, blood was collected from the retro-orbital vein of the rats, and the serum was separated in the cooling centrifuge by centrifuging at 2500 rpm for 10 min.

High Cholesterol Diet-Induced Hyperlipidemia: Hyperlipidemia was induced in rats by giving a high cholesterol diet for 10 days in standard rat chow diet. The drug solution was administered orally. High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2% with standard powdered standard animal food. The diet was placed in the cage carefully and was administered for 10 days [22].

Experimental Procedure

Group 1: Administered vehicle and served as normal control.

Group 2: Fed with atherogenic diet and served as HCD control.

Group 3: Administered pitavastatin 10 mg/kg, p.o., and fed with HCD.

Group 4: Administered lower dose of a chloroform extract (200 mg/kg),p.o., and fed with HCD.

Group 5: Administered higher dose of chloroform. (400 mg/kg), p.o., and fed with HCD.

Group 6: Administered lower dose of methanol extract (200 mg/kg), p.o., and fed with HCD.

Group 7: Administered lower dose of methanol extract (400 mg/kg), p.o., and fed with HCD.

The animals were fed a high-cholesterol diet for 10 days. To confirm the induction of hyperlipidemia, blood samples were collected by retro-orbital vein. The TC concentration of the blood samples was then determined using a standard diagnostic kit. The rats were then divided into 7 groups of 6 animals based on their cholesterol levels, after which the treatments were administered orally once daily for 10 days.

Histopathological Studies: On the day of blood withdrawal, the two animals from each group were sacrificed, and the liver was isolated. Tissue samples were immersed in 10% formalin for at least 24h to fix the tissue. The tissue was embedded in paraffin wax, sectioned and stained with hematoxylin and eosin. The sections were viewed under the light microscope for histopathological changes [23].

Biochemical Lipid Parameters: The main biochemical parameters recommended by the National Cholesterol Education Program (NCEP) guidelines (2002) for lipid screening [24]. Blood was collected on the 7th day (Triton treated) and 20th (HCD) day by retro-orbital puncture technique under mild anesthesia after 8 h fasting and allowed to clot for 30 min at room temperature. Blood

samples were centrifuged at 3000 rpm for 20 min. Serum was separated and stored at -20 °C until biochemical estimation carried out. A serum sample was analyzed spectrophotometrically for total serum cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) Very low-density lipoprotein (VLDL) low-density lipoprotein (LDL-C) was estimated by using diagnostic kit.

Biological Parameters: Biological parameters like body weights were determined just before the animals were sacrificed.

Evaluation of *in-vivo* Antioxidant Activity:

Estimation of Superoxide Dismutase: This method is well described by Mccord and Fridovich (1969) and can be applied for the determination of the antioxidant activity of a sample. It is estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 lL of the lysate, 75 mM of tris-HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol are added. An increase in absorbance is recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by the change in absorbance/min at 420 nm. The activity of SOD is expressed as units/mg protein [25].

Estimation of Catalase: Catalase activity can be determined in erythrocyte lysate using Aebi's method (Aebi, 1984). Fifty microliters of the lysate are added to a cuvette containing 2 ml of phosphate buffer (pH 7.0) and 1 ml of 30 mM H_2O_2. Catalase activity is measured at 240 nm for 1 min using a spectro- photometer. The molar extinction coefficient of H_2O_2, 43.6M cm^{-1} was used to determine the catalase activity. One unit of activity is equal to 1 mmol of H_2O_2 degraded per min and is expressed as units per milligram of protein [26].

Estimation of Glutathione: Glutathione is an intra-cellular reductant and plays a major role in catalysis, metabolism, and transport. It protects cells against free radicals, peroxides, and other toxic compounds (Sapakal *et al*., 2008). Deficiency of GSH in the lens leads to cataract formation. Glutathione also plays an important role in the kidney and takes part in a transport system involved in the re-absorption of amino acids. The method illustrated by Ellman (1959) can be used for the determination of antioxidant activity. The tissue homogenate (in 0.1M phosphate buffer pH 7.4) is taken and added with an equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins.

The mixture is allowed to stand for 5 min before centrifugation for 10 min at 2000 rpm. The supernatant (200 lL) is then transferred to a new set of test tubes and added with 1.8 ml of the Ellman's reagent (5,50-dithiobis-2- nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes are made up to the volume of 2 ml. After completion of the total reaction, solutions are measured at 412 nm against the blank. Absorbance values were compared with a standard curve generated from known GSH [27].

Statistical Analysis: The results obtained in all analysis were expressed in median ± SD (standard deviation). The levels of statistical significance ($P<0.05$) were calculated based one-way ANOVA test for comparisons among means.

RESULTS:

Preliminary Phytochemical Analysis:

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS

Preliminary chemical tests	*Syzygium cerasoideum*			
	Pet-ether extract	**Chloroform extract**	**Ethyl acetate extract**	**Methanol extract**
I. Test for Triterpenoids/Steroids				
Liebermann Burchard Test	+	+	-	+
II. Test for Glycosides				
Keller Killiani Test	-	+	+	+
Legals test	+	+	+	−
Bromine test	+	+	+	+
III. Test for Saponins				
Foam test	-	+	+	+
IV. Test for Alkaloids				
Mayer's test	-	+	+	+

Hager's test	-	+	+	+
		V. Test for Flavanoids		
Ferric chloride test	-	+	+	+
Alkaline reagent test	-	+	+	+
Lead acetate solution test	-	-	-	-
		VI. Test for Tannins/phenols		
$KMNO_4$ test	-	+	-	+
5% $FeCl_3$	-	+	-	+

+ Indicates Presence and - Indicates Absence

TABLE 2: STANDARDIZATION OF EXTRACTS BY HPTLC

Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
0.18 R_f	0.6 AU	0.26 R_f	333.2 AU	100	0.30 R_f	0.3 AU	6724 AU	100	Kaempferol
0.18 R_f	6.5 AU	0.25 R_f	349.5 AU	100	0.28 R_f	1.2 AU	12441.6 AU	100	Kaempferol
0.18 R_f	14.3 AU	0.23 R_f	308.0 AU	100	0.27 R_f	1.0 AU	11809.2 AU	100	Kaempferol
0.24 R_f	0.7 AU	0.27 R_f	54.5 AU	100	0.30 R_f	0.3 AU	1443 AU	100	SC-chloro
0.21 R_f	2.8 AU	0.25 R_f	64.5 AU	100	0.30 R_f	0.1 AU	2597 AU	100	SC-MeOH
0.22 R_f	2.9 AU	0.26 R_f	57.6 AU	100	0.31 R_f	0.5 AU	1694 AU	100	SC-MeOH

Scan@365Table

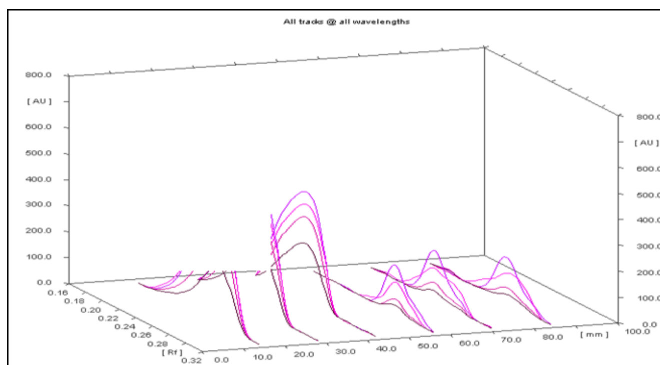


FIG. 1: HPTLC DENSITOMETRIC CHROMATOGRAM OF KAEMPFEROL AND SAMPLE SCC AND SCM AT 365 nm

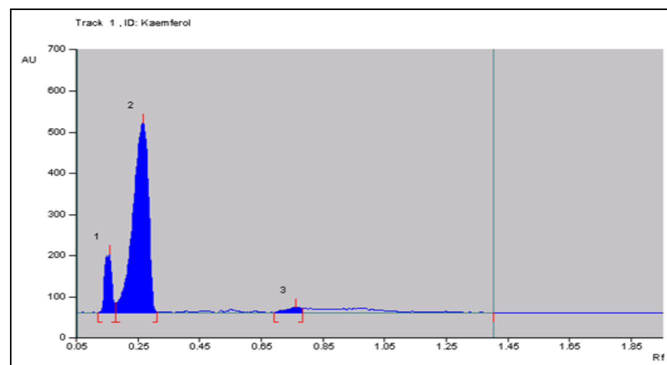


FIG. 2A: DENSITOMETRIC CHROMATOGRAM OF KAEMPFEROL AT 365 nm

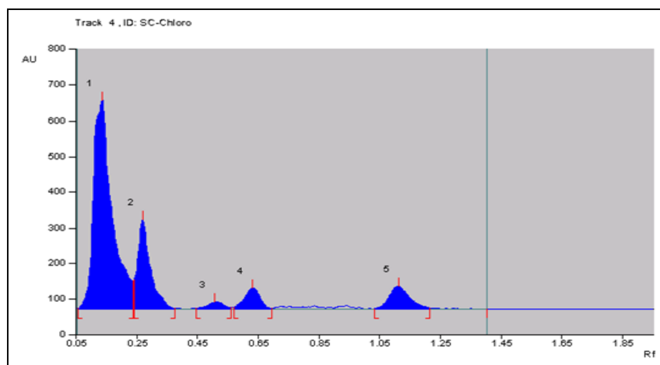


FIG. 2B: DENSITOMETRIC CHROMATOGRAM OF CHLOROFORM EXTRACTS OF SC AT 365 nm

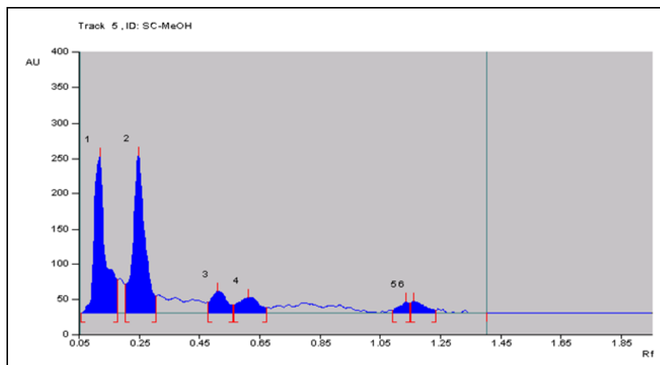


FIG. 2C: DENSITOMETRIC CHROMATOGRAM OF METHANOLIC EXTRACTS OF SC AT 365 nm

Quantification of Kaempferol in Extract SC-Chloroform:
Standard area: 12441.6
Sample area: 1442.9
Sample dilution: 25 mcg/mL
Standard dilution: 1 mcg/mL

The calculation for Content of Kaempferol in SC-Chloroform:

(Sample area)/(Standard area) × (Standard weight)/(Sample weight) × (Sample dilution)/(Standard dilution) × Purity

1442.9/12441.6 × 1/25 × 25/1 × 90 = 10.431 µg/mL

Quantification of Kaempferol in Extract SC-MeOH:
Standard area: 12441.6
Sample area: 2596.6
Sample dilution: 25 mcg/1mL
Standard dilution: 1 mcg/mL

The calculation for Content of Kaempferol in SC-MeOH:

(Sample area)/(Standard area) × (Standard weight)/(Sample weight) × (Sample dilution)/(Standard dilution) × Purity

2596.6/12441.6 × 1/25 × 25/1 × 90 = 18.73 µg/mL

Triton WR 1339 Induced Hyperlipidemia: Effect of administration of chloroform and methanolic extracts of *S. cerasoideum* (200 and 400 mg/kg, p.o., once) pitavastatin (10mg/kg, p.o., once) on serum lipid parameter levels in rat treated with triton (400 mg/kg, i.p., once) measured at 7th day.

High Cholesterol Diet-Induced Hyperlipidemia: Effect of administration of chloroform and methanolic extracts of *Syzygium cerasoideum* (200 and 400 mg/kg, p.o., once) pitavastatin (10 mg/kg, p.o., once) on serum lipid parameter levels in rat treated with HCD measured on the 20th day.

TABLE 3: TRITON WR 1339 INDUCED HYPERLIPIDEMIA

Groups	T. Cholestrol	Triglycerides	HDL	LDL	VLDL
Normal Control	141.33±3.2***	76±4.28***	16.17±0.79***	109.96±3.54**	15.2±0.78***
Hyperlipidemic	268±3.72	209.85±3.15	60.83±4.23	165.2±8.11	41.97±0.63
Standard	129.67±1.31*** (48.38↓)	84±1.63*** (40.03↓)	20.5±1.06*** (33.70↑)	92.37±2.2*** (55.91↓)	16.8±0.33*** (40.03↓)
Chloroform 200 mg/kg	181.17±2.14** (67.60↓)	100.5±2.29** (47.89↓)	19.33±0.67** (31.78↑)	141.74±2.66* (85.80↓)	20.1±0.46*** (47.89↓)
Chloroform 400 mg/kg	168.67±2.3*** (62.94↓)	90.17±2.7*** (42.97↓)	17±0.86*** (27.95↑)	133.63±1.82* (80.89↓)	18.03±0.54*** (42.96↓)
Methanolic 200 mg/kg	136.83±1.66*** (51.06↓)	95.83±1.7*** (45.67↓)	17.17±0.91*** (28.23↑)	100.49±1.79** (60.83↓)	19±0.34*** (45.27↓)
Methanolic 400 mg/kg	121.67±2.81*** (45.40↓)	69.67±0.88*** (33.20↓)	13.33±0.8*** (21.91↑)	94.40±2.36*** (57.15↓)	13.93±0.18*** (33.19↓)

Statistical analysis using one way ANOVA followed by Tukey. Values are expressed as mean ± SEM for six animals P <0.05 is significant when compared with disease control. P<0.001 significant to disease control. The number in the parenthesis indicates % decrease or increase in the respective serum levels. ↓ denotes decrease, ↑ denotes an increase in respective serum levels.

TABLE 4: HIGH CHOLESTEROL DIET-INDUCED HYPERLIPIDEMIA

Groups	T. Cholesterol	Triglycerides	HDL	LDL	VLDL
Normal Control	94.83±1.78***	132.33±2.97***	23.17±1.51***	45.2±3.1***	26.47±0.54***
Hyperlipidemic	204.33±2.32	222.33±3.76	63.17±2.15	102.03±3.8	39.13±0.75
Standard	98.83±2.24*** (48.37↓)	137±2.25*** (61.62↓)	26.67±0.95*** (42.22↑)	44.77±2.47*** (43.88↓)	27.4±0.45*** (70.02↓)
Chloroform 200 mg/kg	140.33±1.17** (68.68↓)	138.33±2.78** (62.22↓)	25.5±1.23** (40.37↑)	87.17±0.76** (85.44↓)	27.67±0.56** (70.71↓)
Chloroform 400 mg/kg	127.17±2.41** (62.24↓)	141.17±2.51** (63.50↓)	39.33±2.6** (62.26↑)	59.6±2.72** (58.41↓)	28.23±0.5** (72.14↓)
Methanolic 200 mg/kg	126.67±1.71** (61.99↓)	132.5±2.28** (59.60↓)	35.67±1.69** (56.47↑)	64.5±2.71** (63.22↓)	26.5±0.46** (67.72↓)
Methanolic 400 mg/kg	104.17±2.95*** (50.98↓)	93.33±4.33*** (41.98↓)	30.83±2.46*** (48.80↑)	54.67±3.54*** (53.58↓)	18.67±0.87*** (47.71↓)

Statistical analysis using one way ANOVA followed by Tukey P<0.05 when compared with disease control significant **P<0.001 and significant*** P<0.001. The number in the parenthesis indicates % decrease or increase in the respective serum levels. ↓ denotes decrease, ↑ denotes an increase in respective serum levels.

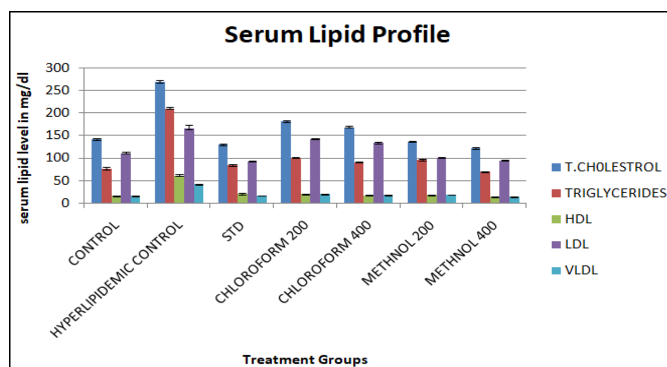


FIG. 3: TRITON WR 1339 INDUCED HYPERLIPIDEMIA

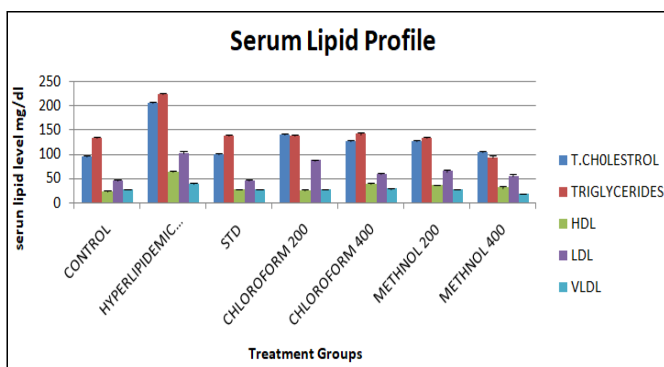


FIG. 4: HIGH CHOLESTEROL DIET-INDUCED HYPERLIPIDEMIA

Antihyperlipidemic Activity: In triton induced hyperlipidemic model, the groups treated with the extracts of *Syzygium cerasoideum* and pitavastatin demonstrated a significant decrease in the levels of TC, TG, LDL-C, VLDL-C particularly treatment with CESC at the dose of 200 mg/kg b.w resulted in significant decrease in levels of TC (67.60%), TG (47.89%), LDL-C (85.80%) and VLDL-C (47.89%) whereas HDL-C levels are significant to standard. In cholesterol induced hyperlipidemic model, the groups treated with the extracts of *S. cerasoideum* and pitavastatin demonstrated a significant decrease with the CESC and MESC at low doses in the serum levels TC (68.68%), TG(60.22%), LDL-C (85.44%), VLDL-C (70.71%) besides an increase in serum HDL-C (62.26%) levels when compared to cholesterol-induced hyperlipidemic control group. The groups treated with the extracts of *Syzygium cerasoideum* and pitavastatin shows a remarkable decrease in the levels of SOD, CAT, and GSH activities when compared to the cholesterol-induced hyperlipidemic control group.

Body Weight Analysis: A significant increase in body weight was detected in HCD feed rats compared to normal control. In the present study, however, no favorable changes in body weight were detected after *Syzygium cerasoideum* extract dosing and particularly with methanolic 400 mg/ kg the body weight shows (41.94%) which show similarity with standard body weight (41.51%).

TABLE 5: BODY WEIGHT ANALYSIS

Days	Mean Body weight (g)						
	Normal	HCD	STD	Meth.400mg	Meth.200 mg	Chl.400 mg	Chl.200 mg
0th day	188.3±4.5	186.7±4.7	186.7±5.2	186.7±4.4	186.7±2.4	185.8±2.7	184.2±3.7
5th day	196.7±4.4 (4.46 ↑)	204.2±2.3 (9.37 ↑)	205±2.2 (9.80 ↑)	204.2±3.0 (9.37 ↑)	203.3±1.6 (8.89 ↑)	204.2±1.5 (9.90 ↑)	204.2±2.0 (10.86 ↑)
10th day	205±2.8 (8.87 ↑)	223.3±1.6 (19.60 ↑)	224.2±2.3 (20.09 ↑)	225±3.1 (20.51 ↑)	225.8±1.5 (20.94 ↑)	228.3±3.0 (22.87 ↑)	227.5±2.8 (23.51 ↑)
15th day	212.5±2.1 (12.85 ↑)	260.8±1.5 (39.69 ↑)	249.2±2.0 (33.48 ↑)	250±1.8 (33.90 ↑)	257.5±2.1 (37.92 ↑)	255.8±3.5 (37.67 ↑)	260.8±2.3 (41.59 ↑)
20th day	230±1.8 (22.15 ↑)	303.3±6.2 (62.45 ↑)	264.2±0.8 (41.51 ↑)	265±1.8 (41.94 ↑)	285±4.2 (52.65 ↑)	276.7±4.5 (48.92 ↑)	291.7±4.7 (58.36 ↑)

***In-vivo* Antioxidant Activity:** Rats treated with extracts of *Syzygium cerasoideum* and pitavastatin caused a significant decrease in the levels of SOD, CAT and GSH activities at the doses of 400 mg/kg/day and 10 mg/kg/day respectively.

TABLE 6: *IN-VIVO* ANTIOXIDANT ACTIVITY

	SOD µg/mg	CAT µg/mg	GSH µg/mg
Normal Control	21.5±0.76***	7.22±0.46***	1.89±0.13***
Hyperlipidemic	10.17±0.4	3.34±0.22	0.98±0.05
Std.	19.76±0.47***	6.41±0.31***	5.31±0.2***
Chloroform 200	11.43±0.28**	4.15±0.23**	2.28±0.2**
Chloroform 400	14.13±0.77***	6.11±0.23***	4.15±0.18***
Methanolic 200	16.15±0.46***	5.63±0.21***	3.52±0.26***
Methanolic 400	18.41±0.4***	7.11±0.1***	5.2±0.09***

P-values *<0.05, **<0.01, ***<0.001.

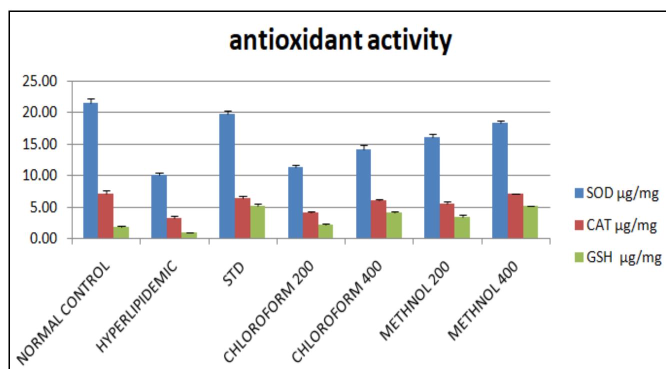


FIG. 5: *IN-VIVO* ANTIOXIDANT ACTIVITY

Histopathological Studies:

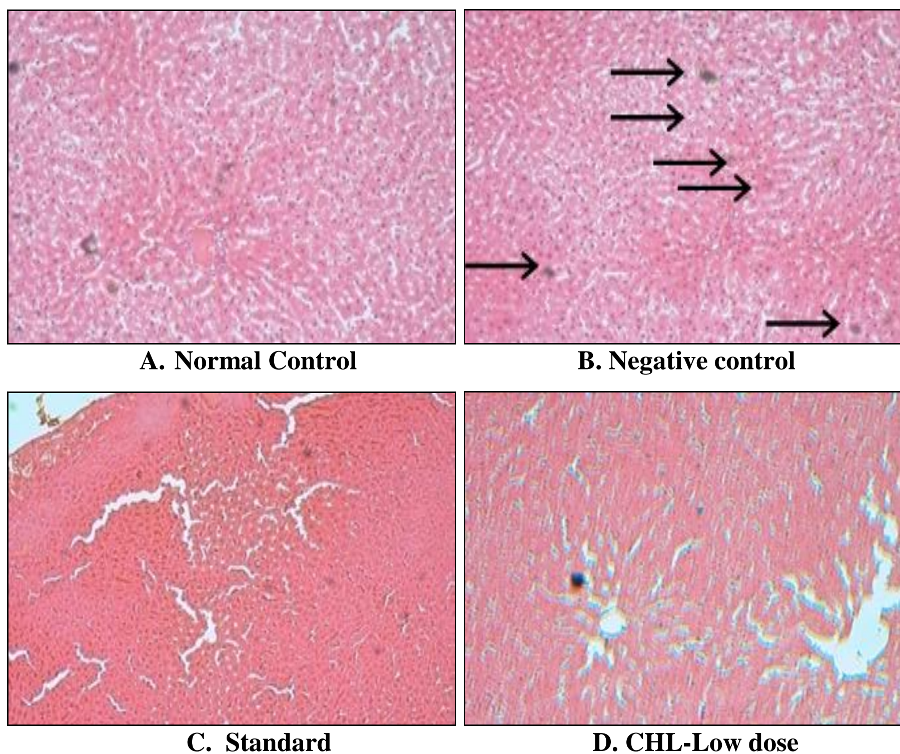
A. In normal sections of the liver shows no fatty changes.

B. Hyperlipidemic control shows Mild to Moderate degree microvesicular fatty changes.

C. The standard section shows No fatty changes

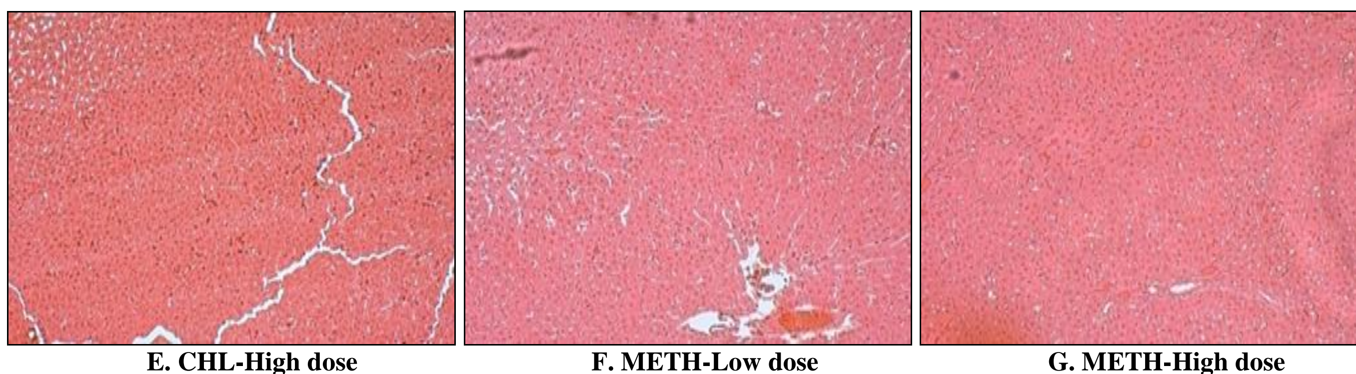
D. Chloroform extracts show No fatty changes compared to normal architecture.

E. Methanolic extracts show No fatty changes compared to normal architecture.



A. Normal Control **B. Negative control**

C. Standard **D. CHL-Low dose**



E. CHL-High dose **F. METH-Low dose** **G. METH-High dose**

FIG. 6: HEPATOCYTES OF RATS STAINED WITH HEMATOXYLIN AND EOSIN (100X MAGNIFICATION). A) Control group showing normal architecture; B) Hyperlipidemic group showing Mild to Moderate degree microvesicular fatty changes; C) Standard section shows No fatty changes; D) No Necrosis or malignant cells seen; E) No Necrosis or malignant cells were seen; F) No Necrosis or malignant cells are seen; G) No Necrosis or malignant cells are seen.

DISCUSSION: Preliminary phytochemical tests revealed the presence of flavonoids, glycosides, phenolic compounds in the extracts. Amount of kaempferol present in the chloroform and methanolic extracts were quantified. Triton WR-1339 has been widely used to block clearance of TG-rich lipoproteins to induce acute hyperlipidemia in several animals. Schurr *et al.*,

demonstrated that parenteral administration of Triton induced hyperlipidemia in adult rats. The large increase in plasma TC and TG due to Triton administration results mostly from an increase of VLDL secretion by the liver accompanied by a noticeable decline in VLDL and LDL catabolism. Triton acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extrahepatic tissues, resulting in increased blood lipid concentration. While the triton model of hyperlipidemia in rodents is widely used for a variety of purposes, the rat model particularly has been used for screening natural or synthetic hypolipidemic drugs. Among the two dose levels tested in the study, 400 mg/kg b.w. proved to be the most effective for hyperlipidemia. MESC at a dose of 400 mg/kg b.w significantly lowered both plasma TG and TC levels.

Several animal and human studies have confirmed the hypercholesterolemic properties of saturated fatty acids and cholesterol, which include increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hyper-cholesterolemia- related metabolic disturbances in different animal models. The mechanism of action of cholic acid is two-fold: an increase in cholesterol absorption and concomitant suppression of cholesterol 7a-hydroxylase activity that results in decreased cholesterol excretion [28].

From the obtained result, it was observed that keeping the animal on HCD significantly increased the TC, TG, LDL-C level in serum ($P<0.05$) as compared to rats on a normal diet. When HCD was co-administered with SC extracts, the elevated levels of TC, TG, and LDL-C condition has shown a considerable decline. It was noted that TC, TG, and LDL-C lowering activity of methanolic extract (400 mg/kg) of SC was more significant as compared to Chloroform extract. There was a significant elevation in plasma HDL-C in SC treated rats as compared to HCD rats, thus indicating the efficacy of SC extract in preventing the elevation seen in various components of lipid profile under experimentally induced hyperlipidemia. Ample of evidence exists concerning the fact that HDL cholesterol is inversely related to total body cholesterol and a reduction of plasma HDL cholesterol concentration may accelerate the development of atherosclerosis leading to ischaemic heart diseases, by impairing the clearing of cholesterol from the arterial wall [29].

Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats [30]. Flavonoids and polyphenols found in our SC extracts could, therefore, be considered favorable in increasing HDL and decreasing LDL and VLDL in CT treated rats. The observed activity of antioxidants may be due to the presence of phenolic phytochemicals in extracts [31].

Which offer a possible role in reducing the oxidative stress by inducing cellular antioxidant enzymes. Flavonoids and polyphenolic compounds are potent free radical scavengers and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules [32]. Most phenolic phytochemicals are believed to function by countering the effects of reactive oxygen species generated during cellular metabolism. Phenolic phytochemicals due to their phenolic ring and hydroxyl substituents similar to that found in vitamin E can function as effective antioxidants due to their ability to neutralize hydroxy and related free radicals. It is, therefore, believed that dietary phenolic antioxidants could scavenge harmful free radicals and thus inhibit their oxidative reactions with vital biological molecules [33] and prevent the development of physiological conditions, which can manifest many harmful diseases [34].

CONCLUSION: In the present study, *Syzygium cerasoideum* showed significant results in experimental hyperlipidemia. Rats were treated with extracts of *Syzygium cerasoideum* and pitavastatin caused a significant decrease in the levels of SOD, CAT and GSH activities at the doses of 400 mg/kg/day and 10 mg/kg/day respectively. The histopathological findings in the liver of rats fed with the SC extracts showed decreased in mild to moderate microvesicular changes caused by HCD. All these beneficial effects of the extract may be due to their antioxidant and antihyperlipidemic effects carried out by polyphenols and related compounds present in them.

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