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FORMULATION AND EVALUATION OF *VERNONIA ELAEAGNIFOLIA* PHYTO-FORMULATION FOR THE MANAGEMENT OF HYPERLIPIDAEMIA

Sneha Nawale *, K. Prasanna Laxmi, M. Sindhu Bhargavi and M. Ganga Raju

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Osmania University, Bachupally, Hyderabad - 500090, Telangana, India.

Keywords:

Antihyperlipidemic activity, *Vernonia elaeagnifolia*, Nano suspension, Triton X-100, Propylthiouracil

Correspondence to Author:
Dr. Sneha R. Nawale

Associate Professor,
Department of Pharmacognosy,
Gokaraju Rangaraju College of Pharmacy, Osmania University,
Bachupally, Hyderabad - 500090,
Telangana, India.

E-mail: sneha.nawale11@gmail.com

ABSTRACT: The aim of the present investigation was to formulate and evaluate phytoformulation prepared from the ethanolic extract of *Vernonia elaeagnifolia*. *Vernonia elaeagnifolia* whole plant was collected and extracted with ethanol by soxhlation. Dried ethanolic extract of *Vernonia elaeagnifolia* was used to formulate the nanosuspension by homogenization method to enhance the bioavailability of phytoconstituents by increasing its solubility. Nano suspension of *Vernonia elaeagnifolia* (NS-VE, 50 mg/kg, 100 mg/kg) was evaluated for particle size, polydispersed index (PDI), entrapment efficiency, zeta potential, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC).NS-VE was screened for antihyperlipidemic activity with propylthiouracil induced and triton X-100 induced hyperlipidemia models in rats. The average particle size of NS-VE was found to be 0.027µm, PDI 60% (0.026 µm), 5mV zeta potential, and 46% entrapment efficiency. NS-VE (at doses 50 mg/kg, 100mg/kg bd.wt *p.o*) showed significant ($p<0.01$) anti-hyperlipidemic effect by reducing levels of TG, TC, LDL, VLDL and shown raised level in HDL when results were compared with normal control, disease control, and standard group. Nanosuspension of *Vernonia elaeagnifolia* can be used for the management of hyperlipidemia and other metabolic disorders with improved bioavailability.

INTRODUCTION: Dyslipidemia is a major cause of atherogenesis and related conditions like coronary heart disease (CHD), ischemic cerebro-vascular disease, and peripheral vascular disease. Dyslipidemia is a condition of elevation in plasma lipids, includes triglycerides (TG), cholesterol, phospholipids along with plasma lipoproteins (VLDL, HDL, and LDL) [1]. Variety of cardio-vascular diseases is one of the major deadly cause of fatality and mortality among the global population, which contribute to nearly one-fourth of the deaths in the age group of 25-65 years [2].

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Based on the Indian Council of Medical Research (ICMR) survey, there is a high dominance of hypercholesterolemia in the civic area as compared to rural area [3]. High lipid levels may result due to enhanced absorption of lipids throughout the gut or improved endogenic synthesis of lipids. Dyslipidemia can be induced by diet management, *i.e.*, high fat diet and with triton X-100 induced hyperlipidemia model [4-9]. Dyslipidemia can be controlled with triton X-100, without change/ manipulation in diet, but by blocking the endogenic synthesis of lipid levels or by decreasing fat absorption from guts. Both these elements can be evaluated in normal animals [10].

Nanosuspension is a technological tool applied mainly to unravel the problem of poor solubility and bioavailability of phytoconstituents and occasionally to improve drug safety and efficacy by

altering their pharmacokinetics. It is used as an alternative approach to lipid systems when the phytoconstituents/drug is insoluble in aqueous and organic media. The reduced particle size of poorly water-soluble drug to nano range enormously increases surface area leading to an increased rate of dissolution or an increase in saturation solubility due to an increased dissolution pressure [11].

In the present study, nanosuspension of *Vernonia elaeagnifolia* ethanol extract was formulated, characterized, and screened for its anti-hyperlipidaemic action.

MATERIALS AND METHODS:

Plant Collection, Identification and Extraction of *Vernonia elaeagnifolia*: *Vernonia elaeagnifolia* whole plant was collected from RR District, Hyderabad, Telangana. Crude plant material was identified and authenticated by Dr. P. Suresh Babu, Botanist, (Voucher specimen no., VEP-6) Government Degree College, Kukatpally Hyderabad (T.S.), and India. *Vernonia elaeagnifolia* crude plant material was cleaned, shade dried and coarsely powdered. The powdered crude material was subjected to soxhlation with ethanol, and crude ethanol extract was dried and stored for further use.

Preparations of Nano-suspension of *Vernonia elaeagnifolia* (NS-VE): [12] Nanosuspension of *Vernonia elaeagnifolia* was prepared by the homogenization method. Ethanolic extract of *Vernonia elaeagnifolia* (0.15g) was dissolved in ethanol by sonication for 10 min, and polyvinyl alcohol (1.5%) was mixed with extract solution. In order to formulate nanosuspension, 0.1% chitosan solution was prepared in water, and phosphoric acid was added to maintain the pH 5.4. Chitosan solution was mixed with extract solution dropwise with sonication (Probe sonicator-HD 2070, Source - Bandelin Sonopuls, Germany) for 30 min. The greenish opalescent nanosuspension of *Vernonia elaeagnifolia* (NS-VE) was formed.

Characterization of Nano-suspension:

Particle Size Distribution, Poly Dispersity Index (PDI) and Zeta Potential: The NS-VE was analyzed for its particle size, PDI (particle homogeneity in the dispersion), and zeta potential using Zetasizer (Nano ZS, Malvern Instruments, Malvern, UK). The particle size was measured by Particle size analyzer (Nanotrac wave, Model: - W-3275, Microtrac, USA) using dynamic light scattering technique [13].

Entrapment Efficiency: The entrapment efficiency of NS-VE was determined by sonicating NS-VE at 20,000 rpm for 20 min. Aliquots of above solutions (0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL and 0.5mL) were taken and diluted up to 10 mL. Resultant concentrations were centrifuged at 1500 RPM for 20 min. The aliquot of the supernatant was measured by UV/visible spectrophotometry at 246 nm[14]. Entrapment efficiency (%) was calculated using this formula:

% Entrapment efficiency = Total amount of drug − Free drug in supernatant × 100 / Total amount of drug

Morphological Analysis by Scanning Electron Microscopy (SEM): [15] Scanning electron microscopy was performed at high magnifications, generates high-resolution images, and precisely measures very small features and objects. The surface morphological characteristics and particle size of NS-VE were carried out using a scanning electron microscope (Hitachi-S 3400N) at an acceleration voltage of 15.0 kV. SEM analysis was done in the Central Analytical facility-University College of Technology, Osmania University, Hyderabad, Telangana, India.

Differential Scanning Calorimetry (DSC): [16] DSC is a productive technique used for the evaluation of the thermal properties of NS-VE. NS-VE was subjected to heating rate of 10°C/min from 25 °C to 300 °C; the heat absorbed or evolved was recorded as exotherm or endotherm. DSC analysis was done in the Central Analytical facility-University College of Technology, Osmania University, Hyderabad, Telangana, India.

Fourier Transform Infrared (FT-IR) Spectroscopy: FT-IR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information. FTIR spectrum was run for NS-VE, and crude extract of *Vernonia elaeagnifolia* on IR affinity 1 spectro-photometer (Shimadzu, Japan) and absorption bands were recorded and expressed in cm^{-1} [17].

Experimental Animals:

Animals: Wistar rats weighing about 170-200 g were procured from Jeeva Life sciences, Uppal, Hyderabad for present experimental study. The

data protocol was approved by the IAEC (Institutional Animal Ethical Committee Reg. No. 1175/PO/Re/S/08/CPCSEA) of CPCSEA (Committee for control and supervision of experimentation on animals).

***In-vivo* Anti-hyperlipidemic Activity of NS-VE Propylthiouracil (PTU) Induced Hyperlipidemia Model:** Wistar rats were randomized into five groups containing six animals each. Group-I served as normal control rats, Group-II disease control rats treated with PTU (10 mg/kg bd. wt), Group-III hyperlipidaemic rats treated with NS-VE (50 mg/kg bd. wt. *p.o)*, Group-IV hyperlipidaemic rats treated with NS-VE (100 mg/kg bd. wt. *p.o)*, Group-V hyperlipidaemic rats treated with Atorvastatin (10 mg/kg bd. wt).

Group-II to Group-V animals were treated PTU (10 mg/kg bd. wt) for 8 days and on 8th day treated with cholesterol (400 mg/kg b. wt). 6 h post-treatment, blood was withdrawn by retro-orbital plexus, and lipid levels were estimated [18].

Triton Induced Hyperlipidemic Rat Model: Wistar rats were randomized into five groups containing six animals each. Group-I served as normal control rats, Group-II disease control rats treated with triton X 100 (100 mg/kg b. wt *i.p*), Group-III hyperlipidaemic rats treated with NSVE (50 mg/kg bd. wt. *p.o*.), Group-IV hyperlipidaemic rats treated with NSVE (100 mg/kg bd. wt. *p.o*), Group-V hyperlipidemic rats treated with standard Atorvastatin (10 mg/kg bd. wt.). On 8th day blood was collected through retro orbital plexus, serum lipid levels were measured using a cholesterol kit (ERBA diagnostics Mannheim GmbH) and the data was analyzed [19]. Histo-pathological study was carried out for rat livers.

Statistical Analysis: Statistical data analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The results were expressed as mean ± SEM using Graph Pad Prism version 8.0 for Windows.

RESULTS: The present research work was aimed to formulate phytoformulation of *Vernonia elaeagnifolia* for the assessment of anti-hyperlipidaemic activity. *Vernonia elaeagnifolia* was extracted by soxhlation with ethanol, and the ethanolic extract was used in the formulation. Nanosuspensions of *Vernonia elaeagnifolia* were prepared by the homogenization method to increase biological activity as secondary metabolites are associated with slow and insufficient absorption with inconsistent bioavailability. Formulated nano-suspension (NS-VE) was characterized for its particle size, particle size distribution, zeta potential, and morphological features with SEM. Their SEM images revealed the particle size of suspension within the accepted range of nano-particles. The average particle size, PDI, and zeta potential for NS-VE nanoformulation were found to be 0.027 nm, 0.046, and + 5Mv, respectively **Fig. 1**.

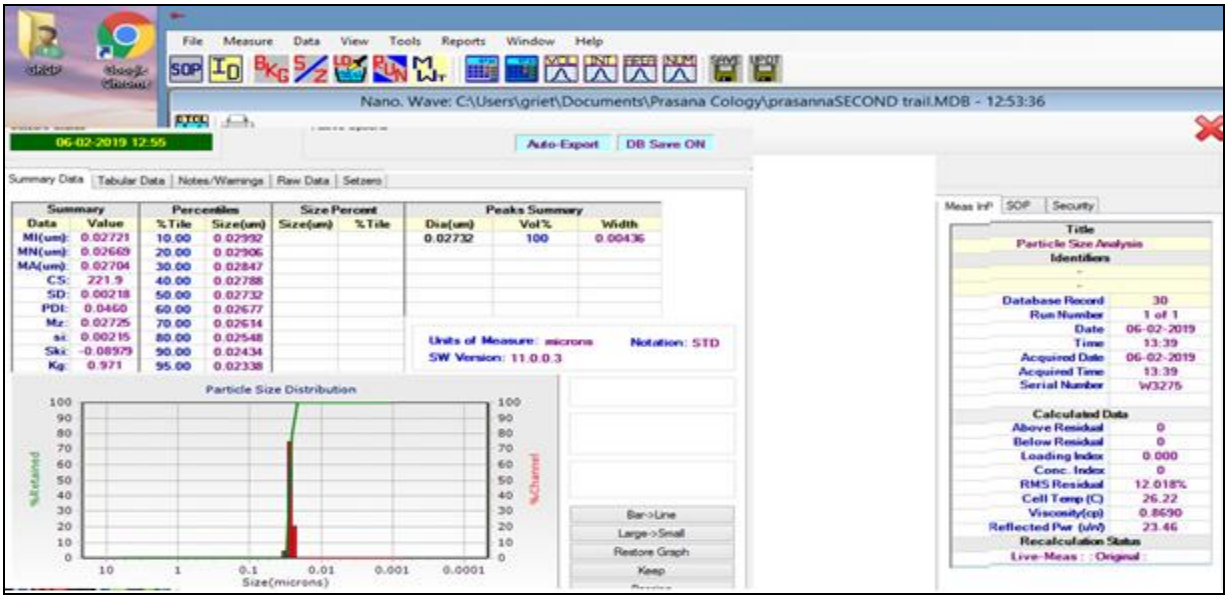

FIG. 1: PARTICLE SIZE ANALYSIS OF NS-VE FORMULATION USING NANOTRAC

The polydispersity index values represent the average uniformity of a particle solution. Large PDI values ranged 0.027 ± 0.04 (60%) represent particle size distribution as well as the stability of the nano-particles. Zeta potential shows the charge on the particle surface, which indicates the physical stability of dispersed systems. Zeta potential of NS-VE was 5mV indicating that the nanoparticles have a little cat-ionic charge due to the presence of chitosan **Fig. 2**.

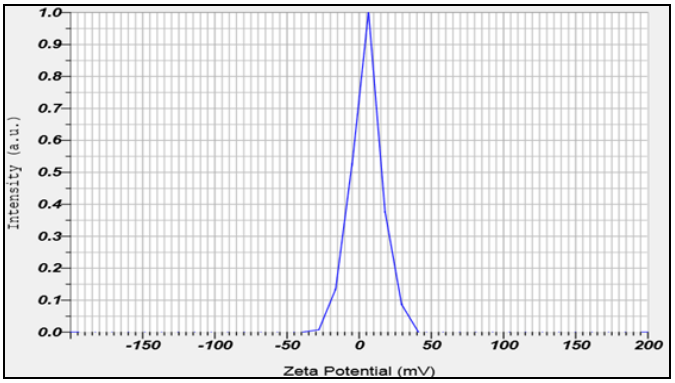

FIG. 2: ZETA POTENTIAL OF NS-VE FORMULATION

Nanonisation of herbal extracts can lead to an increase in dissolution velocity, wetting, particle surface area, and saturation solubility. This may, in turn, bring about more bioavailability due to enhanced *in-vivo* release as only solubilized particles can be absorbed through lipophilic cellular membranes [20]. These nanostructure systems might be able to potentiate the required action of herbal extracts, reducing the necessary dosage, side effects, and improved biological activity.

Scanning electron microscopy was performed for NS-VE. Scanning electron images for surface morphology of NS-VE nanosuspension revealed their smooth texture, spherical shape and smooth topology **Fig. 3**. DSC is a productive technique used for evaluating the thermal properties of NS-VE formulation. The thermogram of NS-VE and *V. elaeagnifolia* **Fig. 4** and **Fig. 5** exhibited endotherm at 100.75 °C and 169.41 °C. This thermogram also showed a broad endotherm between 48.6 °C-108.8 °C and 160 °C - 168 °C corresponding to the melting endotherm of NS-VE and *V. elaeagnifolia* extract. This is maybe due to the conversion of secondary metabolites from crystalline to amorphous form.

The percentage of drug entrapment efficiency was calculated for NS-VE with chitosan polymers and was found to be 46%.

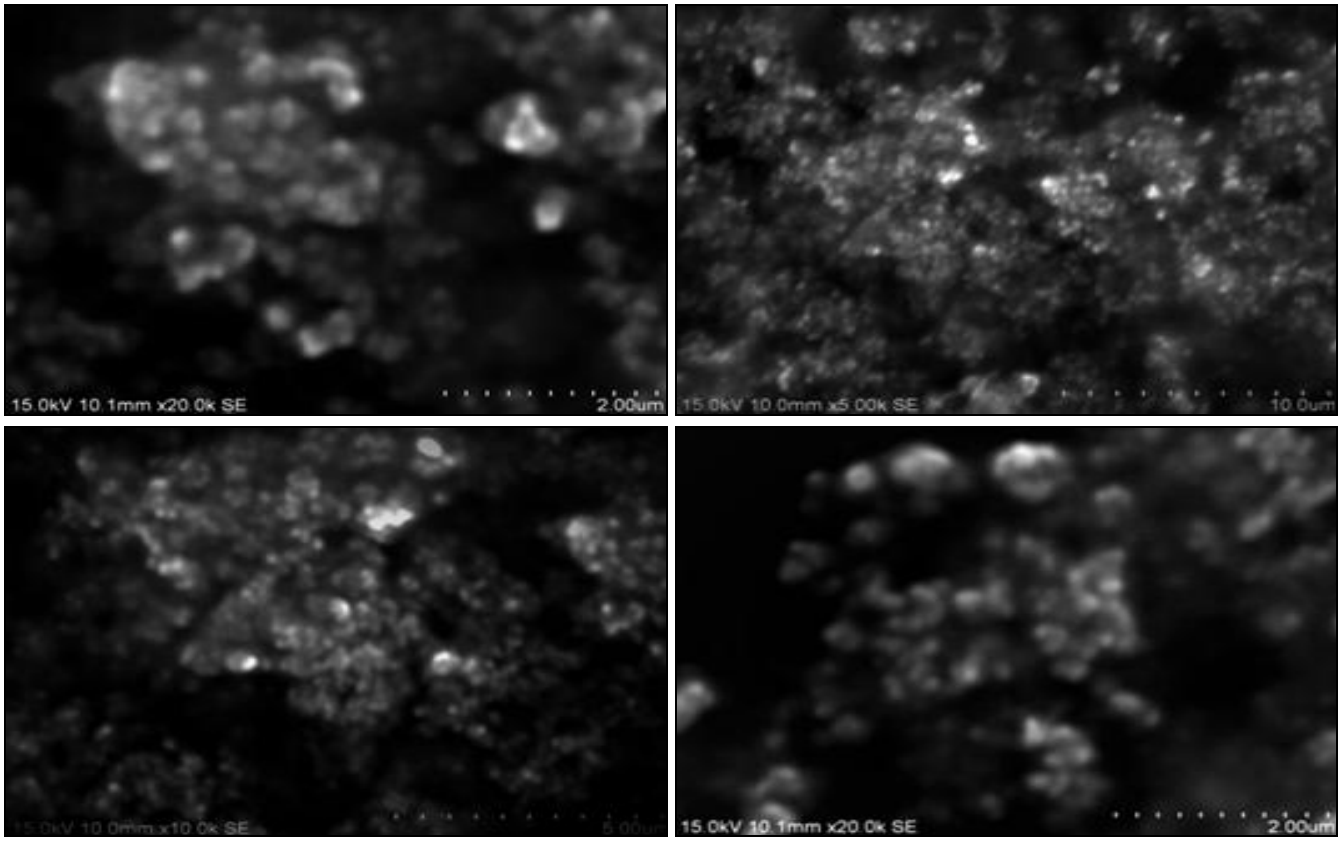

FIG. 3: SEM IMAGES OF NANO SUSPENSION OF *VERNONIA ELAEAGNIFLOIA* EXTRACT (NS-VE)

FTIR spectroscopy of NS-VE shows the sharp characteristics peak at 3500 cm^{-1} (OH), 2848 cm^{-1} (CH), 740 cm^{-1} (C-C), 1456 cm^{-1} (C=C), 1729 cm^{-1} (C=O), 1380 cm^{-1} (CH_3-CH_3) function groups, respectively.

The prominent peaks representing NS-VE and *V. elaeagnifolia* extracted appear in the spectra of NS-VE nanosuspension and did not show any significant shifting in the position of the absorption peak **Fig. 6**.

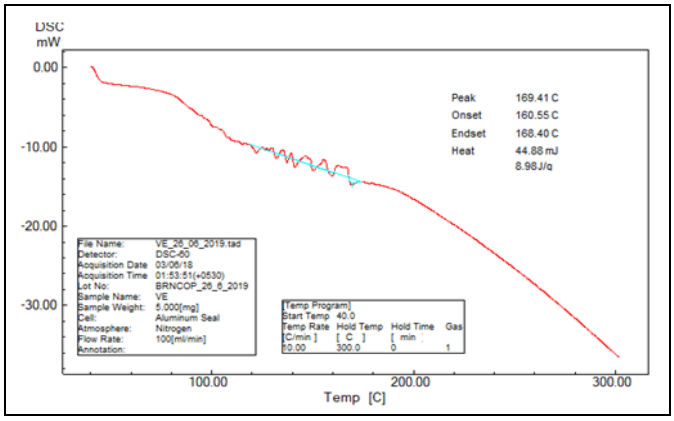

FIG. 4: DIFFERENTIAL SCANNING CALORIMETRY THERMO GRAM OF NS-VE FORMULATION

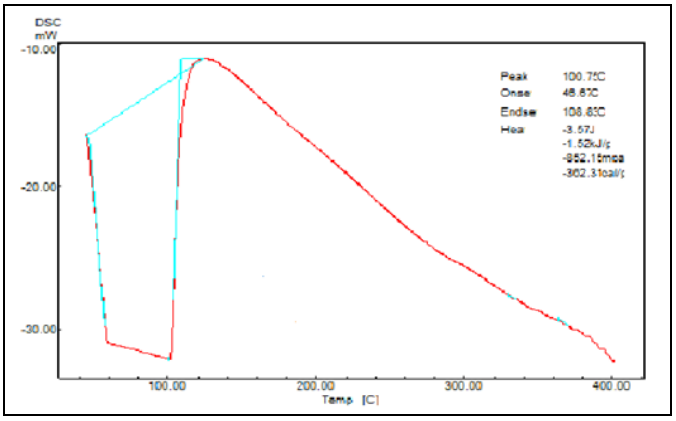

FIG. 5: DIFFERENTIAL SCANNING CALORIMETRY OF *VERNONIA ELAEAGNIFOLIA*

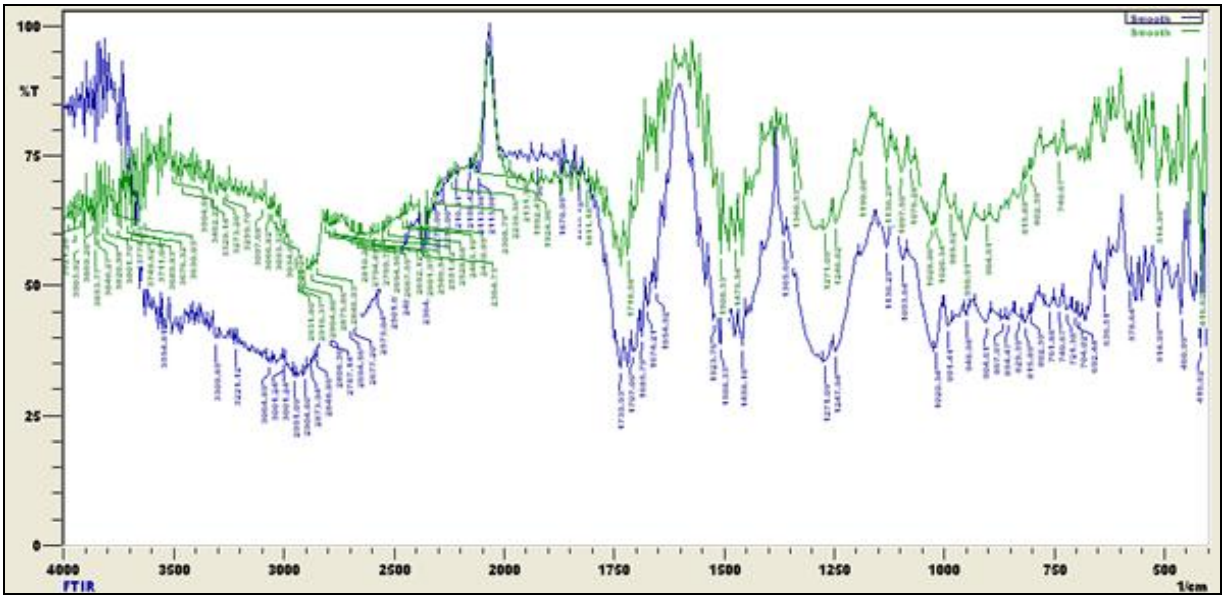

FIG. 6: OVERLAY SPECTRA OF *VERNONIA ELAEAGNIFOLIA* ETHANOLIC EXTRACT AND NS-VE

***In-vivo* Antihyperlipidemic Activity of NS-VE with Propyl Thiouracil Induced and Triton 100 X induced Hyperlipidemic Rat Models:** Nano-suspension of *Vernonia elaeagnifolia* was explored for its antihyperlipidemic activity in propyl-thiouracil induced and triton induced hyper-lipidemic rat models. Administration of PTU and Triton X100 elevated the serum lipid and lipoprotein levels in experimental animals. Hyper-lipidaemic rats were treated with NS-VE (50 mg/kg bd.wt and 100 mg/kg bd.wt.) and standard Atorvastatin (10 mg/kg bd. wt.). The results were compared with normal animals, the disease control group, and standard Atorvastatin, which showed significant improvement in serum lipids and lipoproteins. NS-VE (50 mg/kg bd. wt and 100 mg/kg bd. wt.) treated animals showed significant (P<0.01) decrease in serum TC, TG, LDL, VLDL levels and significant (P<0.01) increased HDL level in PTU and triton 100 X induced hyper-lipidaemia rat models when results were compared statistically with control group, disease control group and standard group **Table 1** and **Table 2**.

Furthermore, the antihyperlipidaemic action of NS-VE was confirmed with histopathological study in triton 100 X rat model **Fig. 7- Fig. 11**.

TABLE 1: EFFECT OF NS-VE ON LIPID LEVELS OF PROPYL THIOURACIL INDUCED HYPERLIPIDAEMIA IN RATS

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	58.6 ± 0.84	107 ± 0.74	37.1 ± 0.60	42.4 ± 0.85	21.4 ± 0.65
Disease control	139.6 ± 0.76	258.6 ± 0.63	17.3 ± 0.49	72 ± 0.73	52 ± 0.32
NSVE (50 mg/kg bd.wt.)	116.6 ± 0.88^{*Aa}	192.3 ± 0.99^{*Aa}	23 ± 0.73^{*Aa}	53.4 ± 0.84^{*Aa}	39.6 ± 0.42^{*Aa}
NSVE (100 mg/kg bd.wt.)	102.7 ± 0.57^{*Aa}	178.1 ± 0.75$^{* Aa}$	28.5 ± 0.42^{*Aa}	34.2 ± 0.68^{*Aa}	31.8 ± 0.89^{*Aa}
Atorvastatin (10 mg/kg bd.wt.)	79.8 ± 0.60^{*A}	138 ± 0.68^{*A}	31.16 ± 0.65^{*A}	23.4 ± 0.59^{*A}	28.6 ± 0.63^{*A}

Values are expressed as Mean ± SEM (n=6). Statistical analysis was performed by using ANOVA, followed by Dunnett's test. (** = $p<0.01$) when compared to the control group, (A = $p<0.01$) when compared to the disease control group, (a = $p<0.01$) when compared to the standard group

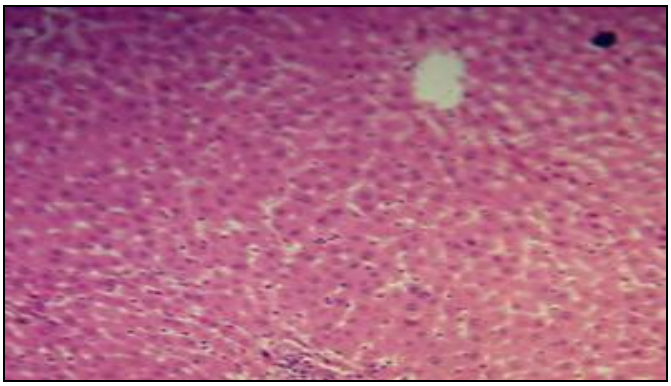

FIG. 7: HISTOPATHOLOGY OF CONTROL RAT: BILE DUCT, KUPFFER CELLS, AND SINUSOIDS APPEAR NORMAL, NO INFLAMMATION OR FIBROSIS NOTICED SURROUNDING THE PORTAL REGION OF LIVER. NO EVIDENCE OF FATTY CHANGE AND FIBROSIS

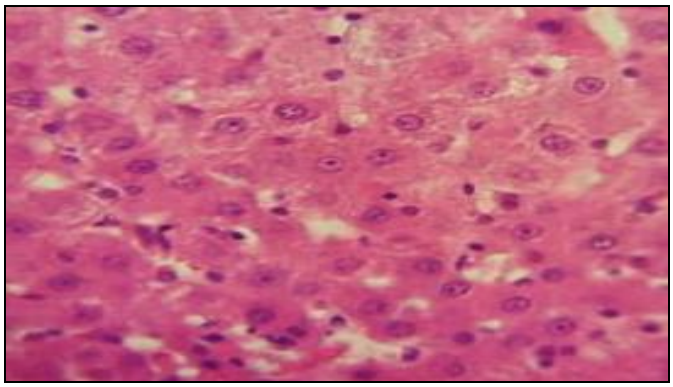

FIG. 8: HISTOPATHOLOGY OF HYPERLIPIDEMIC CONTROL RAT: CORD PATTERN OF HEPATOCYTES, FEW PERIPORTAL LYMPHOCYTES IN FOCAL AREA, FIBROSIS NOTICED IN PERIPORTAL REGION OF LIVER & FATTY CHANGE FOUND IN CYTOPLASM & FIBROSIS

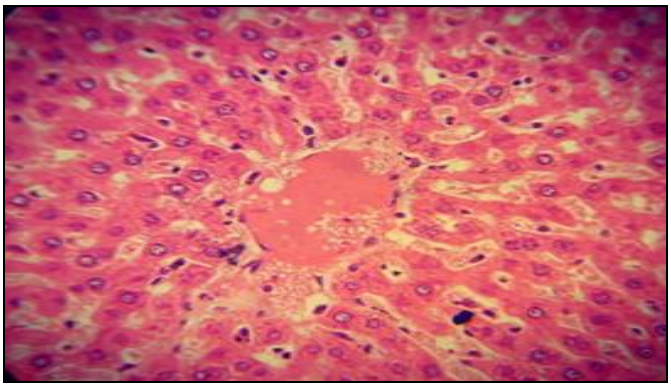

FIG. 9: HISTOPATHOLOGY OF RAT'S LIVER TREATED WITH NS-VE 50 mg/kg bd.wt.: MODERATE SINUSOIDAL SPACE DILATATION ALONG WITH HEMORRHAGES IN THE SINUSOIDAL SPACE OF LIVER AND FEW PERIPORTAL LYMPHOCYTES IN FOCAL AREA

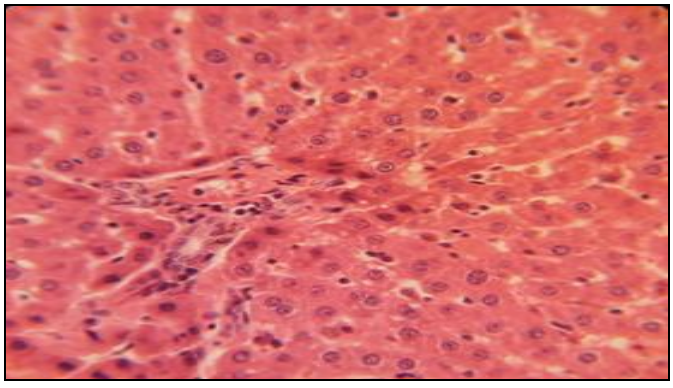

FIG. 10: HISTOPATHOLOGY OF RAT'S LIVER TREATED WITH 100 mg/kg NSVE: MILD CORD PATTERN OF HEPATOCYTES, MILD SINUSOIDAL SPACE DILATION ALONG WITH HEMORRHAGE & NORMAL KUPFFER CELLS

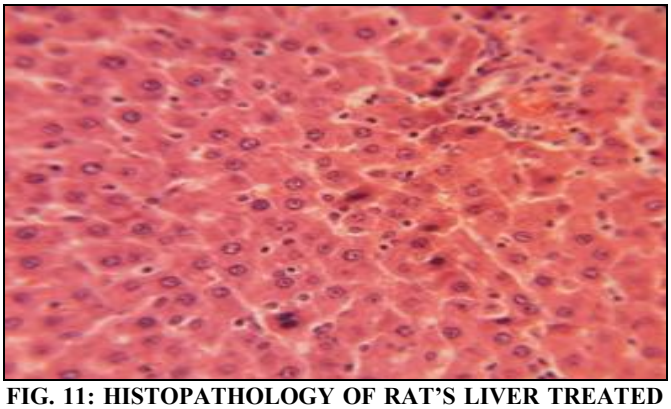

FIG. 11: HISTOPATHOLOGY OF RAT'S LIVER TREATED WITH ATORVASTATIN 10 mg/kg. HEPATOCYTES, KUFFER CELLS AND SINUSOIDS APPEARED NORMAL AND NO EVIDENCE OF FAT DEPOSITION

TABLE 2: EFFECT OF NS-VE ON LIPID LEVELS OF TRITON X-100 INDUCED HYPERLIPIDEMIC RATS

Treatment	Lipid levels (mg/dL)				
	TC	TG	HDL	LDL	VLDL
Normal control	188.33 ± 0.99	129.66 ± 0.87	52.16 ± 0.54	96.5 ± 0.77	24.33 ± 0.96
Disease control	284.83 ± 0.80	204.83 ± 0.92	16.66 ± 0.69	160.33 ± 0.76	74.83 ± 0.98
NSVE (50 mg/kg bd.wt.)	262.5 ± 0.820$^{*A\,a}$	184.33 ± 1.07$^{*A\,a}$	32.8 ± 0.79$^{*\,A\,a}$	22.8 ± 0.7$^{*\,A\,a}$	62 ± 0.66$^{*\,Aa}$
NSVE (100 mg/kg bd.wt.)	238.66 ± 1.88^{*Ans}	164.33 ± 0.83$^{*\,A\,a}$	43.33 ± 0.50$^{*\,A\,a}$	33.33 ± 0.50^{*Aa}	46.33 ± 0.87$^{*A\,a}$
Atorvastatin (10 mg/kg bd.wt.)	230 ± 0.96^{*A}	159 ± 1.1$^{*\,A}$	48.8 ± 0.36^{*A}	38.8 ± 0.36$^{*\,A}$	44 ± 0.91$^{*\,A}$

Values are expressed as Mean ± SEM (n=6). Statistical analysis was performed by using ANOVA, followed by Dunnett's test. (* = p<0.01) when compared with the control group, (A = p<0.01) when compared with the disease control group, (a = p<0.01, ns) when compared to the standard group

DISCUSSION: This study deals with the effect of NSVE extract (50 & 100 mg/kg bd.wt) on plasma and hepatic lipid profile in hyperlipidemic rats. There was a significant reduction in plasma, and hepatic lipid profiles along with elevation in plasma HDL in NS-VE treated rats as compared to hyperlipidemic rats, thus indicating the efficacy of *Vernonia elaeagnifloia* extract in preventing the elevation seen in various components of lipid profile under experimentally induced hyper-lipidemia. Epidemiological studies have shown that a higher level of HDL in plasma reduces the risk of coronary artery disease. Flavonoids are reported to increase HDL concentration and decrease LDL and VLDL levels in hypercholesteremic rats [21-23]. Flavonoids found in *Vernonia elaeagnifloia* extract could, therefore, be considered favorable in increasing HDL and decreasing LDL and VLDL in Hyperlipidemic and NS-VE treated rats.

HMG Co-A reductase is the key metabolic enzyme for the de novo synthesis of cholesterol in the liver; flavonoids are reported to decrease the activity of HMG Co-A reductase [24-25]. Hence, low levels of hepatic TC observed in NSVE treated rats could be due to the inhibition of HMG Co-A reductase activity.

CONCLUSION: A detailed investigation has been initiated to screen the nanosuspension of *Vernonia elaeagnifolia* for its antihyperlipidaemic action. It can be concluded that *Vernonia elaeagnifolia* ethanolic extract has an excellent lipid-lowering potential and possess curative properties in conditions of hyperlipidemia and related disorders. The nano-suspension of *Vernonia elaeagnifolia* has shown significant antihyperlipidemic activity in propylthiouracil induced and triton X-100 induced hyperlipidemia. Administration of nano-suspension of *Vernonia elaeagnifolia* extract has shown a synergistic effect due to its enhanced bioavailability parameter.

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CONFLICTS OF INTEREST: There is no conflict of interest.

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