Research Article

Antihyperlipidemic Potential of Polyphenol and Glycoside Rich *Nerium oleander* Flower against Triton WR-1339-Induced Hyperlipidemia in Experimental Sprague Dawley Rats

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Nerium oleander Linn. (NO), an evergreen cardiac glycoside-rich shrub is used as folklore medicine in China to treat many diseases. It exhibits a wide spectrum of bioactivities but there were not much scientific reports on the bioactivity of *N. oleander* flowers. In the present study, we have evaluated the toxicity profile of the 50% hydroethanolic extracts of *Nerium oleander* flowers (ENO) using *in vitro* brine shrimp lethality assay and MTT cytotoxicity assay and *in vivo* acute toxicity test as per the OECD guidelines. The antihyperlipidemic activity of the ENO was also studied using Triton WR-1339-induced hyperlipemic rats and compared with standard Atorvastatin. *In vitro* brine shrimp, MTT cytotoxic assay, and *in vivo* acute toxicity assays showed a wide safety margin which has been evidenced through its lethal concentration (LC₅₀: 795.46 µg/mL) and growth inhibition (GI₅₀: 993.60 µg/ml) values. Plasma lipids and lipoproteins were significantly elevated by the intraperitoneal injection of Triton WR 1339 in hyperlipidemic rats at 6th and 24th hour. ENO pretreatment showed a significant ameliorative action on elevated lipids and lipoproteins in a dose-dependent manner when compared to standard. Altogether, the results prove that *Nerium oleander* flowers are not toxic at the tested doses and exhibit antilipemic activity.

1. Introduction

World Health Organization (WHO), American Heart Associations, and epidemiologists around the globe have been pronouncing an alarm on the rapid increase in the burden of heart diseases for the past 20 years [1]. Experimental and epidemiological studies have shown that the plasma hyperlipidemic state could contribute to the development of atherosclerosis and cardiovascular-system-related diseases. Cardiovascular diseases (CVDs) are the most common cause of morbidity and mortality in many of the developing countries [2–4]. A logical strategy, to prevent or to treat atherosclerosis and reduce the incidence of cardiovascular disease events, is to target hyperlipidemia either by drugs or dietary intervention [3, 5]. Based on this criterion, a number of plants have received attention and have been shown to lower plasma lipid levels [6]. Earlier studies also

reveal that the consumption of polyphenols and glycoside-rich compounds/plants will reduce the risk of hyperlipidemia [7–10].

Nerium oleander Linn is one such polyphenol- and glycoside-rich evergreen shrub belonging to the Apocynaceae, an important Chinese folk medicine [11]. In early times, it is assumed that all parts of the oleander plant are highly toxic to humans, animals, and certain insects due to the presence of cardiac glycosides mainly nerine and oleandrin at higher concentration [12, 13]. Nowadays, apart from its toxicity, an assortment of pharmacological activities such as antinociceptive, anti-inflammatory, antibacterial, anticancer, CNS depressant activity, antidiabetic, rodenticides, piscicidal, pesticides, insecticides, abortifacients, among others, have been screened and reported in leaves, stems, and roots of *Nerium oleander* except flowers [14–18]. Apart cardiac glycosides, flowers of *Nerium oleander*

contains triterpinoids, ursolic acid, steroids, polysaccharides, among others, reported to have various biological activities [14, 19, 20]. Moreover, our earlier finding confirms the presence of phytochemical constituents in *N. oleander* flower extract (ENO) [21] as well as the cardioprotective and antioxidant potential of ENO against isoproterenol-induced myocardial oxidative damage in experimental rats [22]. These findings prompted us to screen this investigation. Hence, in the present investigation, we have studied the toxicological profile and antihyperlipidemic potential of hydroethanolic extracts of *Nerium oleander* (ENO) flowers in experimental animals.

2. Experimental

2.1. Materials

2.1.1. Drugs and Chemicals. A549 cells were procured from Central Institute for Brackish water aquaculture with passage number 10 as a gift sample. Triton WR 1399 was obtained from Sigma Chemical Co., St. Louis, MO, USA. MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) and DMEM were obtained from Himedia, Mumbai. Atorvastatin standard was obtained from a registered pharmacy, Sri Ramachandra Pharmacy, Chennai, India. Solvents and all other chemicals used were of analytical grade.

2.1.2. Plant Material. About 20 kg of fresh flowers of *N. oleander* (Apocynaceae) was obtained commercially in Chennai, Tamilnadu, and India. It was identified and authenticated by a qualified plant taxonomist Professor V. Jayaraman, Director, Plant Anatomy Research Centre, Tambaram, Tamilnadu, India. The flower was shade air dried and coarsely powdered. About 100 gm of the powder was packed in a Soxhlet extractor with 500 mL of 50% ethanol and extraction was carried out for 18–20 h. The hydroethanolic extract (ENO) of *Nerium oleander* flower was concentrated to dryness under reduced pressure and controlled temperatures (40–50°C) in a rotary flash evaporator. The powdered extract was stored in an air tight container and used for further studies.

2.1.3. Animals. Animal studies were executed after getting approval from the Institutional Animal Ethical Committee (IAEC XV/SRU/106/2009). Male Sprague-Dawley rats (150–250 gm) were obtained and maintained in the Central Animal Facility of Sri Ramachandra University, Porur, and Chennai, India. Experiments were carried out at the Centre for Toxicology and Development Research (CEFT) facility, Sri Ramachandra University, Chennai. Animals were housed in polypropylene cages at a room temperature of $21 \pm 2^\circ\text{C}$ with 12 h light/12 h dark cycles and had free access to standard pellets and water *ad libitum*.

2.2. Methods

2.2.1. Toxicological Profile. The toxicological profile of the hydroethanolic extracts of *Nerium oleander* (ENO) flowers using *in vitro* and *in vivo* methods was studied are as follows.

In Vitro Brine Shrimp Lethality Assay. Brine shrimp lethality bioassay was carried out according to the principle and protocol previously described by Nkengfack et al., [23]. In the experiment, 0.5 mL of the various concentration of ENO (1.5–1000 $\mu\text{g}/0.5\text{ mL}$) in triplicates was added to 4.5 mL of brine solution and maintained at room temperature for 24 h under the light and the surviving larvae were counted. Experiments were conducted along with control (vehicle treated). The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} (concentration at 50% lethal effect) values were determined from the 24 hours count using the best-fit line probit analysis method described by Finney [24].

In Vitro Cytotoxicity Study: MTT Assay. The MTT assay was performed to study the antiproliferative effect of ENO using the earlier protocol [25, 26]. Lung adenocarcinoma cells (A549 Cells) were treated with different concentrations of ENO (100–900 $\mu\text{g}/\text{mL}$) for 24 h. At the end of the treatment, media from control and drug-treated cells were discarded. 20 μL of MTT containing DMEM (0.5 mg/mL) was added to each well. Cells were then incubated at 37°C for 4 h in a CO_2 incubator. Spectrophotometrical absorbance of the purple blue formazan dye was measured using an ELISA reader (BIORAD) at 570 nm. The average values were determined from the triplicate readings, and the average value was subtracted from the blank. The MTT reagent yields low background absorbance values in the absence of cells. Optical density of each sample was compared with control optical density and graphs were plotted. The plot of the data obtained in absorbance against number of cells provides a curve with a linear portion. Thus, both stimulation and inhibition of cell proliferation was measured. GI_{50} (50% growth inhibition) values were determined from the maximum percentage of inhibition:

$$(\%) \text{ Inhibition} = \left[\frac{(\text{Abs. of control} - \text{Abs. of test})}{\text{Abs. of control}} \right] \times 100. \quad (1)$$

In Vivo Acute Oral Toxicity of ENO. The acute toxicity studies were performed in female Sprague Dawley rats as per the OECD guidelines-423. Acute toxicity was performed for three dose of ENO (50, 300, and 2000 mg/kg b.wt). The drug was suspended in 0.5% w/v sodium carboxyl methyl cellulose (CMC) and was prepared freshly each time. After the initial weights (150–200 g) were taken, the animals were distributed randomly into four groups ($n = 3/\text{group}$) for conducting the study. Group I animals receiving the vehicle (0.5% CMC) served as controls and Group II, III, and IV receiving ENO at 50, 300, and 2000 mg/kg b.wt, respectively. After a 12 h fast, the drugs were administered by intragastric route to all the animals.

Animals were observed individually after drug dosing for signs and symptoms such as ataxia, tremors, sleep, coma, among others, at 0, 0.5, 1, 2, 4, 6 hr (with special attention) and then once a day for the next 14 days. Observations also included changes in respiratory, circulatory, autonomic and central nervous systems, as well as somatomotor activity (clinical findings such as ataxia, tremors, convulsion, hair loss, salivation, diarrhoea, lethargy, lacrimation, sleep, coma, etc.) and behaviour pattern. After drug administration, followed by measurement of body weight once in a week, any mortality and behavioral screenings were recorded for 24 h for the next 14 days. The results are expressed as mean $\pm$ SEM of three animals.

2.2.2. Antihyperlipidemia Triton WR-1399-Induced Hyperlipidemia. Hyperlipidemia was induced in experimental rats using Triton WR 1339 by following the earlier method [27–29]. Triton (Superinone, Triton WR-1339), is a polymer of p-isooctylpolyoxyethylenephenol and formaldehyde, a non-ionic surfactant. Aqueous solution of Triton WR 1399 was made in normal saline and injected at a dose of 250 mg/kg., i.p. The test drug ENO doses 10, 30, and 100 mg/kg were selected as per the results obtained in our earlier studies [21, 22]. Atorvastatin (ATV) tablet was used as a standard at a dose of 30 mg/kg. All the drugs were suspended in 0.5% w/v sodium carboxyl methyl cellulose (CMC) and were prepared freshly each time. The drugs were administered by intragastric route.

Experimental Design. After the acclimatization period, animals were divided into nine groups of six rats each: Group I: Normal, Group II: Triton induction, Group III: Triton induction plus ATV (30 mg/kg/day, for 2 weeks) pretreated group, Group IV, V, VI: Triton induction plus ENO (10 mg/kg/day, for 2 weeks) pretreated group, ENO (30 mg/kg/day, for 2 weeks) pretreated group, ENO (100 mg/kg/day, for 2 weeks) pretreated group respectively, Group VII: ENO (100 mg/kg/day, for 2 weeks) alone treated group. After pretreatment with ENO and ATV for a period of 2 weeks, the animals were kept in wire-floored cages throughout the day to limit coprophagia. They were starved for 24 hours before the experiment but allowed free access to water [30]. Blood samples were collected from overnight fasted rats after 1 hr after the last test drug treatment to respective groups for 0th day.

After 1 hr of initial blood collection (0 hr) from respective groups, they were intraperitoneally injected with Triton WR 1339 (250 mg/kg) except Group I and VII animals. The body weight of the rats was individually recorded weekly once. Feed and water were changed every day. At the end of 6, 24, and 48th hr after injection of Triton WR-1339, all the rats were anesthetized for the blood collection. Blood was collected through sino-orbital puncture using EDTA as anticoagulant and the plasma separated was stored at -80°C and used for the lipid and lipoprotein estimation.

Biochemical Analysis—Lipid profile on 0th, 6th, 24th, and 48th Hours. Total cholesterol (TC—Catlog number CL-2009-05-001) and triglycerides (TG—Catlog and TG-2009-05-001)

levels in plasma of 0th, 6th, 24th, and 48th hours were performed with enzymatic method of Accurex kit, Accrex Biomedical Pvt. Ltd., Thane, India using semiautomated biochemical analyzer (Star 21 Plus autoanalyser, Rapid Diagnostics).

HDL-cholesterol concentrations were quantified by the same method as used to determine total cholesterol after removal of other lipoproteins by precipitation with HDL precipitant (Accurex kit Catlog and HD-2009-05-001). The LDL and VLDL cholesterol was calculated by the Friedwald formula [31]:

LDL-Cholesterol

$$= (\text{total cholesterol} - [\text{HDL-Cholesterol} + \text{triglycerides} * 0.16]) \quad (2)$$

VLDL cholesterol = Triglycerides * 0.16.

2.2.3. Statistical Analysis. All data are reported as mean $\pm$ standard error mean (SEM). Statistical analysis was done using Graph pad prism 4.0 for windows package. One-way analysis of variance (ANOVA) was performed followed by post-hoc Tukey's test. The $P < 0.05$ and $P < 0.01$ values were set to access significant protection in treatment groups.

3. Results

3.1. Toxicological Profile. Figure 1 illustrated the *in vitro* toxicity profile of the ethanolic extract of *N. oleander* flower which was performed using *Artemia nauplii* (brine shrimp). Based on the ability of ENO to kill the laboratory-cultured brine shrimp, the toxic nature of ENO was identified. Maximum mortalities were obtained at a concentration of 1000 $\mu\text{g/mL}$ whereas; low mortalities were seen at 30 $\mu\text{g/mL}$ concentration. LD_{50} values were obtained from the best-fit line by plotting concentration verses percentage lethality in probit analysis.

Figure 2 gives an antiproliferative effect of ENO at various doses (10–900 $\mu\text{g/mL}$) against A549 cells (lung adenocarcinoma cell line) for 24 h using MTT assay. ENO exhibits a dose-dependant inhibitory effect on the growth of lung carcinoma cells and the growth inhibition of the cells ($R^2 = 0.958$) is directly proportional to the dose. ENO exhibits greater cytotoxic effect against A549 cell at 900 $\mu\text{g/mL}$ concentration with a GI_{50} value of 993.60 $\mu\text{g/mL}$.

The *in vivo* toxicity profile of ENO was monitored after single oral administration of ENO to female rats at three doses 50, 300, and 2000 mg/kg b.wt, ($n = 3/\text{dose}$) for a period of 14 days. ENO at all doses did not show any remarkable change in the body weights of the rats (data not shown). There were no treatment-related mortality and clinical findings such as ataxia, tremors, convulsion, hair loss, salivation, diarrhoea, lethargy, lacrimation, sleep, coma, among others (data not shown). All experimental rats were found to be normal throughout the study. The LD_{50} of the ENO was found herein to be greater than 2000 mg/kg b.wt when administered once orally via gastric intubation in 12 h fasted female Sprague Dawley rats.

TABLE 1: Effect of ENO and atorvastatin on total cholesterol and triglycerides in plasma of triton administered rats.

Treatments	T. cholesterol (mg/dL)			Triglycerides (mg/dL)		
	6th hr	24th hr	48th hr	6th hr	24th hr	48th hr
Normal Control (NC)	110.12 ± 5.53	104.12 ± 5.12	83.30 ± 3.44	43.46 ± 2.38	41.46 ± 1.98	57.02 ± 4.25
Triton (250 mg/kg, i.p)	137.77 ± 10.90 [†]	260.13 ± 9.48 ^{††}	92.30 ± 13.43	153.88 ± 14.03 ^{††}	431.30 ± 36.26 ^{††}	101.02 ± 5.95 ^{††}
ATV (30 mg/kg, p.o) + Triton	101.70 ± 5.70 ^{**}	207.48 ± 8.32 ^{**}	78.55 ± 10.22	100.76 ± 12.27	240.25 ± 25.39 ^{**}	73.60 ± 7.12 [*]
ENO (10 mg/kg, p.o) + Triton	96.94 ± 5.04 ^{**}	238.04 ± 11.41	85.25 ± 8.77	130.49 ± 17.05	257.04 ± 19.58 ^{**}	68.22 ± 7.65 ^{**}
ENO (30 mg/kg, p.o) + Triton	90.94 ± 6.08 ^{**}	234.04 ± 11.55 [*]	83.25 ± 10.22	127.83 ± 16.81	249.04 ± 16.85 ^{**}	62.22 ± 7.59 ^{**}
ENO (100 mg/kg, p.o) + Triton	112.17 ± 5.33 [*]	236.10 ± 17.03	80.84 ± 11.91	141.59 ± 10.83	257.28 ± 13.27 ^{**}	59.06 ± 4.76 ^{**}
NC + ENO (100 mg/kg, p.o)	88.21 ± 4.11	86.92 ± 3.72	82.24 ± 3.92	33.75 ± 2.00	33.19 ± 1.89	34.18 ± 2.26

The results are expressed in mean ± SEM ($n = 6$); statistical analysis was done using prism 4.0 version, one-way ANOVA, tukey, and P values normal control versus triton-treated rats and NC + ENO-treated rats—[†](0.05) and ^{††}(0.01), triton-treated rats versus triton + atorvastatin and triton + ENO-treated rats—^{*}(0.05) & ^{**}(0.01).

FIGURE 1: Brine shrimp lethality bioassay-probit analysis of ENO. Different concentrations of ENO (1–1000 µg/mL) was tested against brine shrimp for 24 h.

3.2. Antihyperlipidemic Potential of ENO. In the current study, the antihyperlipidemic potential of ENO was also tested using male SD rats. Body weight of the rats was found to be insignificant in all the experimental groups (data not shown). The level of total cholesterol, triglycerides, HDL, LDL and VLDL at 0th, 6th, 24th, and 48th h of the triton induced and ENO treated animals were examined in plasma and all the results are shown in Tables 1 and 2, respectively. The levels of total cholesterol, triglycerides, LDL, and VLDL index were found to be normal at 0th hour of all experimental animals before triton induction (Data not shown).

However, they were found to be significantly ($P < 0.01$) high at 6th h after triton induction in the triton-induced untreated animals with concomitant decrease in HDL when compared to normal control animals. Similarly, at 24th h, the levels of total cholesterol, triglycerides, LDL, and VLDL were found to be at peak with decrease in HDL whereas at 48th h they were found to be nearer to normal in triton-induced untreated animals.

FIGURE 2: Cytotoxic effect of ENO on growth inhibition using A549 cells. Different concentrations of ENO (100–900 µg/mL) was tested against A549 cells for 24 h. The values are Mean ± SEM of triplicates ($n = 3$).

Rats pretreated with ENO (10, 30, and 100 mg/kg) for 14 days significantly prevented the changes in lipid and lipoprotein levels in the plasma after triton induction compared to triton-induced untreated animals. The reduction in the total cholesterol, TGs, LDL, and VLDL levels by ENO was found to be in a dose-dependent manner and they were found to be comparable with Atorvastatin. Alternatively, the levels of lipid and lipoproteins in ENO (100 mg/kg) alone treated rats were found to be insignificant when compared to normal rats.

4. Discussion

Herbal drugs have been the integral part, in one form or another, of several indigenous therapeutic systems including traditional medicine and Indian System of Alternative Medicine [32]. Many numbers of medicinal plants and their active constituents play a role in the prevention and treatment of metabolic disorders like coronary heart disease and diabetes [33–35]. In traditional medicine, various parts of *N. oleander* have been used as a cardioprotective agent [36], rodenticides, piscicidal, pesticides, insecticides, and also used as remedies for indigestion, fever, ringworm, malaria, leprosy, venereal diseases, and as abortifacients [14]. In early times, all parts of the oleander are believed as poisonous to living things but off-late a number of pharmacological activities have been studied and reported by researchers [15]. Any compound to be considered as a drug toxicity studies

TABLE 2: Effect of ENO and atorvastatin on lipoproteins in plasma of triton administered rats.

Groups	HDL-C (mg/dL)			LDL-C (mg/dL)			VLDL (mg/dL)		
	6th hr	24th hr	48th hr	6th hr	24th hr	48th hr	6th hr	24th hr	48th hr
Normal Control (NC)	60.70 ± 3.35	62.70 ± 4.39	41.75 ± 4.29	58.11 ± 2.83	48.06 ± 5.09	51.67 ± 1.75	8.69 ± 0.48	8.29 ± 0.4	11.40 ± 0.85
Triton (250 mg/kg, i.p)	37.13 ± 6.62 [†]	40.67 ± 5.46	25.75 ± 1.81	131.42 ± 11.08 ^{††}	288.47 ± 15.41 ^{††}	82.71 ± 11.75 ^{††}	30.78 ± 2.81 ^{††}	86.26 ± 7.25 ^{††}	20.20 ± 1.19 ^{††}
ATV (30 mg/kg, p.o) + Triton	46.60 ± 2.68	148.48 ± 12.38 ^{**}	30.95 ± 4.84	65.25 ± 6.54 ^{**}	97.44 ± 12.70 ^{**}	59.38 ± 7.37	20.15 ± 2.45 [*]	48.05 ± 5.08 ^{**}	14.72 ± 1.42 [*]
ENO (10 mg/kg, p.o) + Triton	36.71 ± 2.72	110.95 ± 13.60 ^{**}	44.45 ± 7.3	86.33 ± 5.16 ^{**}	168.22 ± 18.53 ^{**}	51.72 ± 8.48	3.17 ± 0.41 ^{**}	51.41 ± 3.92 ^{**}	13.64 ± 1.53 ^{**}
ENO (30 mg/kg, p.o) + Triton	42.71 ± 4.02	136.95 ± 10.51 ^{**}	44.45 ± 8.30	73.80 ± 5.99 ^{**}	136.94 ± 23.06 ^{**}	48.76 ± 8.86 [*]	25.57 ± 3.36 [*]	49.81 ± 3.37 ^{**}	12.44 ± 1.52 ^{**}
ENO (100 mg/kg, p.o) + Triton	70.53 ± 6.01 ^{**}	140.62 ± 13.67 ^{**}	48.27 ± 7.01	69.95 ± 4.39 ^{**}	136.64 ± 28.17 ^{**}	42.02 ± 4.70 ^{**}	28.32 ± 2.17 [*]	51.46 ± 2.65 ^{**}	11.81 ± 0.95 ^{**}
NC + ENO (100 mg/kg, p.o)	48.79 ± 4.12	46.61 ± 4.97	50.54 ± 2.74	46.17 ± 2.96	45.62 ± 2.90	37.17 ± 6.55	6.75 ± 0.40	6.64 ± 0.38	6.84 ± 0.45

The results are expressed in mean ± SEM (*n* = 6); statistical analysis was done using prism 4.0 version, one-way ANOVA, tukey and *P* values normal control versus triton-treated rats and NC + ENO-treated rats—[†](0.05) and ^{††}(0.01), triton-treated rats versus triton + Atorvastatin and triton + ENO-treated rats—^{*}(0.05) and ^{**}(0.01).

is imperative. Hence, assessment of the toxicity profile of *N. oleander* flower was considered a necessity.

In the present study, both *in-vitro* and *in-vivo* toxicity studies were done to identify the LD₅₀ value of ENO and at which dose it is found to be nontoxic. Brine shrimp lethality bioassay which is considered as a useful tool for the assessment of toxicity of herbal extracts [37], in the present investigation the degree of lethality was found to be directly proportional to the concentration of the extract. The LD₅₀ was found to be 795.46 µg/mL which reveals it is reduced toxicity against brine shrimps. Similarly, ENO exhibits a dose-dependant inhibitory effect against the growth of lung carcinoma cells A549 cells. The MTT cell proliferation assay measures the cell proliferation rate and conversely, the reduction in cell viability. The *in vitro* results concluded that the drug at lower concentrations is found to be safer but at higher concentrations it exhibits cytotoxic effect. This cytotoxic and antiproliferative effect of ENO at higher concentrations might be due to the presence of cardiac glycosides which further confirms its anticancer activity [38]. In a similar manner, ENO-treated animals were found to be normal throughout the study in *in vivo* acute toxicity study and its LD₅₀ was found to be >2000 mg/kg b.wt. These results summarize that ENO possess a wide safety margin which has been evidenced through its LD₅₀ and GI₅₀ values.

Though there was a large class of hypolipidemic drugs available worldwide for the treatment, none of them is fully effective, absolutely safe, and free from side effects [39]. Hence, efforts are being made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiovascular diseases. Earlier, we have studied the cardioprotective and antioxidant potential ENO against isoproterenol-induced myocardial oxidative stress, ENO exhibits better free radical scavenging potential and cholesterol reducing activity [22]. Further, to validate the antihyperlipidemic property of ENO, triton WR-1339, a nonionic surfactant, was used in this study to induce hyperlipidemia in experimental animals and compared with standard atorvastatin, a potent lipid-lowering agent which inhibits an early step in the biosynthesis of cholesterol, thereby reducing the plasma cholesterol concentration. Their higher level may decrease the levels of triglycerides along with the cholesterol [40].

Stanley [41] suggested that intravenous or intraperitoneal injection of Triton WR 1339 increases hepatic cholesterol synthesis by increasing HMG CoA reductase activity, the first committed enzyme of the HMG-CoA reductase pathway in rodents within 24 hours which resulted in the increased plasma cholesterol and TG concentrations for upto 36 h. Our results are in corroboration with that of previous reports [28, 42]. Triton causes structural modifications in the circulatory lipoproteins and suppress the action of lipases especially lipoprotein lipase activity and as a consequence block the uptake of circulating lipids by extra hepatic tissues, and, in turn, resulting in increased blood lipid concentration [29]. Significant increase in the level of cholesterol in the triton-induced animals might also be due to the increased activity of HMG CoA reductase.

Triton induction was also reported to interact preferentially with HDL, changing the size and density of lipoproteins which used as substrates for the enzyme LCAT, enzyme activity decreased in parallel to the displacement of apo A-1 [43]. In the present study, reduction in HDL-C in triton-induced animals is agreeable with the earlier statement. In our study, the changes in plasma lipid and lipoprotein levels induced by triton WR-1339 can be resisted by ENO (10 and 30 mg/kg) and atorvastatin (30 mg/kg) pretreatment during triton induction. Substantially, ENO treatment boosted the HDL levels at doses of 10, 30, and 100 mg/kg which might be through the inhibition of the activity of HMG CoA reductase and the stimulation of LCAT activity. Similarly, ENO results were found to be comparable with atorvastatin. Atorvastatin is a competitive inhibitor of HMG-CoA reductase, our results corroborate with this statement.

Moreover, ENO was found to be rich in glycosides and polyphenols as evidenced by its concentration and characterization in our earlier investigations [21, 22]. Glycosides and polyphenolic compounds are reported to inhibit HMG CoA reductase activity [44–46]. Likewise, flavonoids may decrease the risk of cardiovascular disease by increasing the two ratios HDL/TG, HDL/LDL which may hasten removal of cholesterol from peripheral tissues to liver for catabolism and excretion [7, 9].

5. Conclusion

In conclusion, the results of the present study demonstrated that the ethanolic extract of *N. oleander* flower (ENO) was found to be safer at lower concentrations. The antihyperlipidemic activity of ENO is strengthened by its phytoconstituents and can be considered as a potent lipid-lowering and antioxidant agent. These beneficial activities may contribute to its cardio protective and antiatherosclerotic property.

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