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Research Article

Phytochemical Analysis, Antioxidant and Anti Hyperlipidemic Activity of *Nyctanthes arbor-tristis* in Male Wistar Rats

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Abstract

The current study's objective was to assess the antihyperlipidemic, in vitro antioxidant, and qualitative and quantitative phytochemical analysis of *Nyctanthes arbor-tristis* flowers hydroalcoholic extract at 200 and 400 mg/kg in rats that were made hyperlipidemic by a high-fat diet. The Folins Ciocalteau reagent and the aluminium chloride method were used for the quantitative measurement of flavonoids and phenolics, respectively. Through phytochemical investigation, alkaloids, carbohydrates, glycosides, tannins, phenolic compounds, triterpenoids, and steroids were found in the hydroalcoholic extracts of *N. arbor-tristis* flowers. The hydroalcoholic extract flowers had a total phenolic content of 126.000 mg/gm, which was followed by flavonoids at 103.104 mg/gm. Hydroalcoholic extracts of flowers showed concentration-dependent antioxidant activity in vitro against DPPH and superoxide radical scavenging test technique. In this investigation, rats were given a high-fat diet as part of an experimental induction procedure. By lowering LDL and VLDL cholesterol levels and raising HDL levels, the HANAT therapy and the medication atorvastatin dramatically reduce body weight. grew When hydroalcoholic flower extracts were given orally to animals that had been forced to become hyperlipidaemic due to a high cholesterol diet, the levels of HDL cholesterol, triglycerides, low density lipoproteins, and very low-density lipoproteins were all much lower. The p value ($p<0.001$) indicated that the results were significant. The triton-induced study results indicate that animals treated with HANAT at dose levels of 200 mg/kg and 400 mg/kg had considerably lower blood lipid parameters ($p<0.01$) than the control group; however, the 400 mg/kg of HANAT group animals showed significantly higher serum lipid parameters ($p<0.001$) than the control group. Additionally, a higher HDL level was noted at this time.

Keywords: *Nyctanthes arbor-tristis,* Physicochemical analysis, Antioxidant activity, DPPH. Antihyperlipidemic activity

INTRODUCTION

A single unpaired electron causes the production of highly reactive oxygen species (ROS), which in turn causes oxidative stress and is a major factor in the pathophysiology of many physiological conditions, such as ageing, cancer, hepatic, neurological, cardiovascular, and renal disorders[1,2]. Reactive oxygen radicals are produced by environmental pollutants, radiation, chemicals, poisons, deep-fried and spicy foods, as well as physical stress[3]. These radicals cause aberrant protein synthesis and the subsequent depletion of antioxidants within the immune system. Numerous natural antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase, can deactivate free radicals and preserve normal cellular processes[4]. However, dietary antioxidants can be required if endogenous antioxidants are insufficient to sustain adequate cellular activities under elevated oxidative stress[5]. Natural diets high in phenolic and flavonoid compounds that have antioxidant activity have sparked attention in nutrition and food science in recent decades[6]. Plant secondary metabolites with an aromatic ring containing at least one hydroxyl group are known as naturally occurring phenolic and flavonoid chemicals[7]. Because of their ability to directly contribute to antioxidant action through their hydroxyl groups, phenolic substances are effective electron donors[8]. Moreover, a few of them promote the cell's natural production of antioxidant molecules[9]. Numerous studies in the literature state that phenolic substances suppress free radicals, break down peroxide, inactivate metal, scavenge oxygen, and lessen the burden of oxidative illness in biological systems[10]. Leafy vegetables are a great source of natural antioxidants that help shield the body from the damaging effects of free radicals[11]. Consuming green plant vegetables, which contain phenolic and flavonoid chemicals with strong antioxidant activity, has been linked to a lower incidence of diabetes, cancer, heart disease, and neurological illnesses, according to numerous epidemiological studies[12]. The primary risk factor for cardiovascular diseases (CVD) is elevated plasma concentrations of different lipids and lipoprotein fractions, which is the hallmark of the heterogeneous collection of conditions known as hyperlipidemia. Triglycerides, phospholipids, cholesterol, and cholesterol esters are some of these lipids. Large "lipoproteins" called lipids are carried by the blood and have been identified as the leading cause of death in both industrialised and developing countries. It is thought that lipid problems linked to hyperlipidemia are the cause of atherosclerotic cardiovascular disease. Reducing the risk of ischemic heart disease or the development of further cardiovascular diseases such as atherosclerosis or cerebrovascular illness is the primary goal of treatment for people with hyperlipidemia. Many adverse effects have been linked to the medications that are now on the market. Nowadays, there is a global surge in the use of complementary and alternative therapies, particularly in the use of

phytochemicals. Because herbal remedies are more compatible and cause less harm than synthetic pharmaceuticals, they can increase a patient's tolerance even when used for extended periods of time[13-17]. Examining the rich history of traditional medicine is crucial given the growing interest in adopting, researching, and using traditional methods based on various health care systems throughout the world. We have chosen to investigate the N. arbor-tristis in this regard. Indian folklore claims that this shrub is generally employed for religious purposes and is prized for its distinct scent[18]. *N. arbor-tristis* grows well as a shrub up to 3000 feet in height in hilly areas up to 1500 feet above sea level. The shrub reaches a height of 10 metres during its flowering season, July through October. Its leaves are tough and contain stiff, white hair. They feature an orange-colored tube that is 6 to 8 mm long, equal limbs, white lobes that are unequal, and cuneate obcordates. Their calyx is 6 to 8 mm long, and they have a glabrous corolla that is longer than 13 mm. Additionally, the plant produces opposing, simple leaves that are 6-12 cm long[19,20]. The following chemical active components are found in *N. arbor-tristis* leaves. A few significant phytochemical components that have been identified thus far are flavanol glycosides, D-mannitol, β-sitosterol, astragaline, nicotiflorin, oleanolic acid, nyctanthic acid, ascorbic acid, and tannic acid, among others. In India, *N. arbor-tristis* is primarily found south of Godhavari and in the Himalayan regions. Similarly, it is extensively dispersed over Bangladesh, South-East Asia, the Indo-Pakistan peninsula, and Sub-Tropical South East Asia[21]. Key components: β-monogentiobioside of crocin-1 and crocin-3, glucose, nyctanthin, D-mannitol, tannin, β-D monoglucoside ester of α-crocetin, and carotenoids. Arbortristoside A and B, glycerides of linoleic acid, stearic acid, nyctanthic acid, 3-4 secotriterpene acid, oleic acid, lignoceric acid, and a water-soluble polymer composed of D-mannose and D-glucose are all present in the seeds of this plant. *N. arbor-tristis* has alkaloids and glycosides in its bark. From the plant's stem, naringenin-4-0-β-glucapyranosyl-α-xylopyranoside and β-sitosterol have been extracted. Several significant phytoconstituents, including terpenoids, anisaldehyde, phenyl acetaldehyde, and various ketones, are present in flower oil[21]. Among other significant phytoconstituents, it also includes polysaccharides, phenyl propanoid glycoside, nyctanthoside A, nyctanthic acid, friedelin, oleanolic acid, and iridoid glycosides, arbortristosides A, B, and C[22]. The pharmacological actions of *N. arbor-tristis* are extensive throughout the entire plant. For example, the leaves have anti-inflammatory, anti-fungal, antibacterial, and anti-pyretic properties. They are also used as cholagogues, diaphoretics, purgatives, and diuretics. Usually, the decoction of leaves is used to treat hepatic disorders, biliary scattering, and persistent fever. In Ayurvedic medicine, leaf decoction is also used to treat inflammation of the joints and digestive illnesses. The other plant parts, the flowers, have strong antioxidant, diuretic, and anti-filarial properties. They are also utilised in the perfume industry. While the stem and bark have outstanding antimicrobial, antipyretic, and antioxidant properties, the seeds have antifungal, antibacterial, and immunomodulatory properties[18]. This study looked at the phytoconstituents found in the flowers and evaluated their antioxidant qualities. The extract from the plant has a strong ability to decrease cholesterol in diet-induced hyperlipidemia, which explains some of the plant's claimed medicinal benefits.

MATERIAL AND METHOD

Plant material

Newly blooming *N. arbor-tristis* gathered from Sagar, MP locality. The specimen voucher number assigned was BOT/H/03/74/234. Dr. Pradeep Tiwari, a professor in the Department of Botany at Dr. HS Gour University Sagar, (M.P.), created and authenticated the herbarium file of the plant component. Following that, the department received the herbarium file.

Chemical reagents

The following materials were obtained from SD Fine Chemicals Pvt. Ltd. in Mumbai, India: petroleum ether, ethyl acetate, methanol, sodium carbonate, potassium ferricyanide, DMSO, NaOH, ferric chloride, and trichloroacetic acid. The following ingredients were purchased from Sigma Aldrich Chemicals Pvt. Ltd. in Hyderabad, India: gallic acid, rutin, folin-ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitro blue tetrazolium (NBT), triton, and ascorbic acid. The remaining chemicals utilised in this investigation were procured from Hi Media Laboratories Pvt. Ltd., Lobo Chem, Ltd., SRL Pvt. Ltd., and Merck Life Sci. Private Ltd. located in Mumbai, India. Rats were used to measure their blood cholesterol levels using a commercial detection kit called Span diagnostic kit that was purchased from Surat, India. The auto analyzer Star 21 Plus Biochemistry was utilised to estimate various cholesterol levels. All other chemicals used in this study were obtained commercially and were of analytical grade and triple distilled water was used for whole experiment was generated in house.

Processing of plant

The plant material (flowers) chosen for the study were carefully cleaned under running water from the faucet and then rinsed with distilled water. They were then left to air dry at room temperature for a while. After that, the plant material was safely shade dried for three to four weeks. An electronic grinder was used to ground the dried plant material. Colour, flavour, texture, and odour of powdered plant material were noted. Dry plant material was kept for phytochemical and biological research after being sealed in an airtight container.

Extraction

Plant material was extracted for the current investigation utilising the Soxhlet apparatus and a continuous hot percolation process. *N. arbor-tristis* powder (250 gm) was added to a soxhlet apparatus thimble. At 60°C, soxhlation was carried out with petroleum ether serving as a non-polar solvent. After drying, the exhausted plant material (marc) was extracted again using a hydroalcoholic solvent (methanol: water; 70:30%). Each solvent's soxhlation was continued until no discernible colour change was seen in the syphon tube, and the extraction's completion was verified by the lack of any solvent residue upon evaporation. A Buchi-type rotating vacuum evaporator was used to evaporate the obtained extracts at 40°C. Weighing the dried extract, we calculated the % yield for each extract using the following formula:

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labeled till further use[23,24].

Solubility

Dimethyl sulfoxide (DMSO), ethanol, tween 20, tween 80, normal saline (0.9 percent sodium chloride in water), and distilled water were used to examine the solubility of each extract.

Quantitative Analysis of Phytochemicals by Spectrophotometer

Spectrophotometer quantification of total phenolic content

Prepare varying gallic acid concentrations (20–100µg/ml) in methanol. Prepare the test sample in 100µg/ml of methanol or a solvent with nearly the same polarity. Two millilitres of Folin-Ciocalteu Reagent (1:10 in deionized water) and four millilitres of sodium carbonate solution were added to 0.5 millilitres of various concentrations of gallic acid/test sample. With periodic shaking, incubate for 30 minutes at room temperature. Using methanol as a blank, measure the absorbance at 765 nm (because of the acquired blue hue). Create a standard curve for each gallic acid concentration and determine the regression line. Place the test sample's absorbance on the gallic acid standard curve's regression line. Gallic acid equivalent (µg/mg) or mg/gm was used to express the total phenolic content[24,25].

Spectrophotometer quantification of total flavonoid content

Prepare varying concentrations of rutin in methanol (20–100µg/ml). Prepare the test sample in 100µg/ml of methanol or a solvent with nearly the same polarity. Blend a 0.5-ml portion of suitably diluted sample solution with 2 millilitres of distilled water, followed by 0.15 millilitres of a 5% NaNO2 solution. Add 0.15 ml of a 10% AlCl3 solution after 6 minutes, let it stand for another 6 minutes, and then mix in 2 ml of a 4% NaOH solution. Add water right away to make the mixture a final volume of 5 millilitres. Mix the mixture well and let it stand for an additional fifteen minutes. Compare the mixture's absorbance at 510 nm to a produced methanol blank. Create a standard curve for each Rutin concentration and determine the regression line. Place the test sample's absorbance on the rutin standard curve's regression line. The amount of total flavonoid content was determined and reported as mg/gm or µg/mg comparable to rutin[26].

Evaluation of *in-vitro* Antioxidant Activity

Antioxidants are known to stop or slow down the oxidation of substrates mediated by free radicals, which helps shield the body from oxidative stress and related degenerative illnesses[27]. Antioxidants can therefore be given externally to boost the body's own antioxidant defence mechanism. Antioxidants can function through various mechanisms and at varying degrees of oxidation. By lowering oxygen concentrations, scavenging free radicals like hydroxyl radicals to stop chain reactions from starting, or binding to free metal ions to stop metal-induced free radical formation and consequent oxidative damage, they can all lessen oxidative damage[28,29]. Rich sources of phenolic and polyphenolic chemicals can be found in natural antioxidants. In order to further utilise the phytochemical content of herbal plants for the conditions linked to the disease, their antioxidant potential was assessed. The current study aimed to evaluate the extract's potential as an antioxidant and its ability to scavenge free radicals with that of ascorbic acid, a synthetic form of vitamin C, in relation to reactive oxygen species.

1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The assay method for DPPH (1, 1-diphenyl-2-picrylhydrazyl) relies on the reduction of DPPH, which is a stable free radical. The highest absorption of the odd-electron free radical DPPH occurs around 517 nm, giving rise to a purple hue. Antioxidants can cause decolorization (yellow colour) in proportion to the number of electrons captured when they react with DPPH, a stable free radical that pairs off with a hydrogen donor (such as an antioxidant that scavenges free radicals) and is reduced to the DPPHH[30]. As a result, absorbance decreases from the DPPH radical to the DPPH-H form. The decreasing ability increases with decolorization. The most widely used model for assessing a novel drug's capacity to scavenge free radicals is this test. The technique was used to calculate the DPPH radical scavenging activity[31,32]. One millilitre of a 0.1 millilitre DPPH (4 milligrammes per hundred millilitre) in methanol solution was combined with one millilitre of various extract concentrations. After giving the reaction mixture a good vortex, it was allowed to sit at room temperature for half an hour in the dark. As a reference standard, ascorbic acid was utilised, and methanol served as the control. The ability of extracts to function as antioxidants was measured by the reduction of the stable DPPH radical. Using methanolic solution as a reference solution, the UV-MINI-1240, SHIMADZU spectrophotometer was used to measure the colour change at a wavelength of 517 nm. This has to do with how well the control group absorbed the plant extracts. Every test was run in three duplicates. Despite the fact that the activity is stated as 50% inhibitory concentration (IC50), the percentage of DPPH radicals scavenged was used to determine IC50. The level of antioxidant activity increases with decreasing IC50 value. Using the following formula, the percentage inhibition of the free radical DPPH was determined:

% inhibition = [(absorbance of control- absorbance of sample)/absorbance of control] × 100%

(DPPH) + H-A→DPPH-H + A

(Purple) (Yellow)

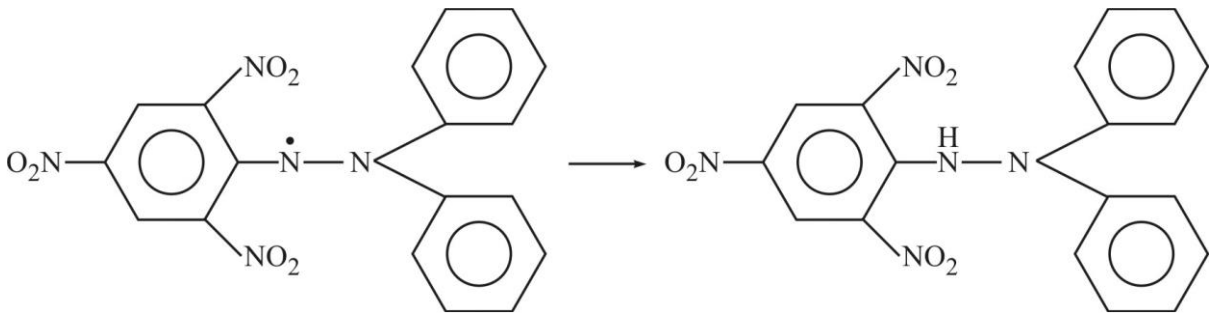

Figure1: Reduction of DPPH

Superoxide radical scavenging assay

The photoreduction of riboflavin produced superoxide radical (O2), which was then eliminated using the nitro blue tetrazolium dye (NBT) reduction technique. The ability of various extract fractions to scavenge super oxide radicals was reported[33] in relation to their ability to prevent the formation of formazan when nitro blue tetrazolium (NBT) was reduced photochemically. To put it succinctly, every 3 ml reaction mixture contained 0.5 ml extract/CuSO4 solution, 130 mM methionine, 60 μM riboflavin, 0.5 mM EDTA, and 0.01M phosphate buffer (pH 7.8). After six minutes of exposure to fluorescent light, the absorbance of these tubes was measured at 560 nm. Superoxide radicals are produced via the nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system, reducing NBT to a purple formazan. Superoxide radicals in the reaction mixture can be quenched by the plant extract and the reference component ascorbic acid, as seen by the drop in absorbance at 560 nm. As blanks, identical tubes were stored in the dark. Percent inhibition was used to express the results in relation to the control.

Superoxide radical scavenging activity (%) = $[(Ao-A_1)/A_o \times 100]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of extract or the standard sample.

Animals

Wistar albino male wistar rats weighing 180-200g were kept in groups of six in controlled temperatures and humidity levels (25±2 °C, 55-65%) with a typical 12-hour light/dark cycle. Water was available at all times, along with conventional rat feed. Prior to doing the trials, the rats were given seven days to become used to the lab environment. Every experiment was conducted from 8:00 to 15:00 in a quiet room. Each series of trials had a different group of six rats. The Ministry of Environment and Forests, Government of India, New Delhi, India, established the Institutional Animal Ethics Committee (IAEC) to oversee and regulate the use of experimental animals. The IAEC granted approval for the animal experiments.

Acute toxicity studies

The Organisation for Economic Co-operation and Development (OECD) protocol for acute oral toxicity was followed[34]. Before administering a dose, animals fast (food should be withheld overnight, but water should not be). Subsequently, the test drug is delivered and the animals are weighed. The rats that were in good health were separated into four groups. Using a curved, ball-tipped stainless steel feeding needle, the test material is given orally as a single dose. In this work, the decoction (p.o.) was administered at doses of 5, 50, 300, and 2000 mg/kg to four groups of six rats each. Three hours are spent without eating after the medication is administered. The animals are monitored for any signs of toxicity and mortality for the first two hours after treatment, then every day for up to 14 days after that, and then on occasion for up to six hours. Changes were detected in the autonomic effects (salivation, lacrimation, gauntness, and piloerection), central nervous system (gait, tremors, and convulsion), and skin and fur, eyes, and mucous membrane (nasal).

Table1: Acute toxicity study design

GROUP	Number of Animals	DOSE (mg/kg)
Group 1	6	5
Group 2	6	50
Group 3	6	300
Group 4	6	2000

Clinical observation

For four hours following dosage, every animal was closely observed for any indications of toxicity. For the following 14 days, more observations are made in case there are any additional behavioural or clinical toxicity indicators. Weight changes are calculated. At the end of the test animals are weighed. The formula is used to determine LD50 values[35].

Dose Calculation Equation

$$\mathbf{LD_{50} = higher\ dose - \Sigma\ (a \times b)/n}$$

Where, **a =** dose difference, **b =** animal died, **n =** No. of animals in each group

$$\mathbf{ED_{50} = \underline{LD}_{50}}$$

Antihyperlipidemic Activity

High-cholesterol diet model

High fat diet induced hyperlipidemic model preparation of feed

A modified version of[36] was used to create hyperlipidemia brought on by a high-fat diet. Typical animal feed pellets were broken up into tiny pieces using a mortar and pestle, and then they were ground into a fine powder using a mixer grinder. In the mixer grinder, the remaining ingredients cholesterol 2%, citric acid 1%, sugar 40%, and coconut oil 10% were added in ascending order of quantity and thoroughly combined. The dried powder was then combined with the same amount of water each time to form little feed balls, which were subsequently refrigerated at 2°C to 8°C under plastic covers that sealed themselves. The usual group's feed was made in a similar way, with just the normal food pellets ground and water mixed in instead of any additional excipients. For every animal, this meal preparation was done once every three days. For thirty days, the animals were fed a diet heavy in fat. By assessing the rats' serum lipid and lipoprotein levels, hyperlipidemia was verified.

Experimental designs

Wistar rats weighing 180-200gm were divided into 5 groups of 6 animals each.

Group I- served +as normal control and were given only vehicle (distilled water)

Group II- received high fat diet served as hyperlipidemic control

Group III - received atorvastatin 10mg/kg served as standard drug

Group IV- received (200mg/kg, p.o.) HANAT

Group V- (400mg/kg, p.o.) HANAT

Estimation of weight gain

Rats' consumption of a high-cholesterol meal and weight growth were noted during the experiment on days 0, 14, and 28 of the HANAT therapy. The approximately thirty grammes of pre-weighed food pellets were inserted into the cage's hopper. Weighing the food remained in the hopper allowed us to determine how much food each rat had eaten.

Biochemical analysis of serum

Following administration with the test medication for 29 days, the rats were kept fasting until day 30, at which point blood was extracted via retroorbital sinus puncture while the rodents were under a light anaesthesia. Serum samples from the obtained samples were utilised for a variety of biochemical assays after they were centrifuged for 10 minutes at 2000 r.p.m. Using commercial kits in accordance with manufacturer

instructions, serum triglycerides (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), and very low density lipoproteins were measured[37-39].

Estimation of lipids

A. Total cholesterol: An Ecoline Diagnostic Kit was used to estimate the amount of cholesterol in the serum. Detergents caused the release of cholesterol and its esters from lipoprotein. The esters are hydrolyzed by cholesterol esterase. H2O2 was produced during the enzymatic oxidation process by cholesterol oxidase. This underwent a peroxidase-catalyzed reaction with 4-aminoantipyine and phenol to become a coloured quinine mine. At 546 nm, the absorbance of the standard and the sample were measured in relation to the reagent blank value. The serum cholesterol level was reported as mg/dL.

B. Triglycerides: The Ecoline Diagnostic Kit was used to estimate the serum level of triglycerides. At 546 nm, the absorbance of the standard and the sample were measured in relation to the reagent blank value. The measurement for serum triglyceride content was mg/dL.

C. HDL cholesterol: Following the precipitation of LDL cholesterol using a phosphotungstic acid precipitating reagent, the cholesterol was extracted from the serum. Ecoline Diagnostic Kits were utilised to quantify the supernatant following centrifugation. At 546 nm, the absorbance of the standard and the sample were measured in relation to the reagent blank value. Serum HDL cholesterol is measured in milligrammes per deciliter.

D. LDL cholesterol: LDL cholesterol was calculated by using the formula

LDL cholesterol = Total cholesterol – [HDL cholesterol – Triglycerides/5].

LDL cholesterol level in plasma was expressed as mg/dL.

E. VLDL cholesterol: VLDL cholesterol was calculated by using the formula

VLDL cholesterol = Total cholesterol – HDL cholesterol – Triglycerides- LDL.

Data were statically analysed as mean + SEM and expressed as just significant $P<0.05$ and significant $P<0.01$ as the case may be using one way ANOVA followed by Dunnett's multiple comparison test[40-42].

RESULTS

In phytochemical extraction, the percentage yield of extraction is crucial for assessing the standard extraction efficiency of a given plant, different sections of the same plant, or different solvents. Using a rotary vacuum evaporator, the extracts were evaporated after the flower portion of the powdered *N. arbor-tristis* was separated separately using a Soxhlet device. Table 2 shows the yield of extracts made from the *N. arbor-tristis* flower using hydroalcoholic as a solvent. The results showed that the floral yield of *N. arbor-tristis* had an extract yield of 3.2% in petroleum ether and 7.8% in hydroalcoholic. Standard assays were used in qualitative phytochemical testing of extracts to investigate the presence or lack of different phytochemical elements. The hydroalcoholic extract of *N. arbor-tristis* was subjected to phytochemical testing. Phytochemical analysis of hydroalcoholic flower extracts from *N. arbor-tristis* revealed the presence of triterpenoids, steroids, alkaloids, polysaccharides, and glycosides in addition to tannins and phenolic compounds. Chemical assays were used to identify the phytoconstituents and revealed the presence of several constituents. Table 3 displays the outcomes of the *N. arbor-tristis* flower extract. As a result, the extracts chosen for the pharmacological investigations. Using gallic acid as a reference, the total phenolic content of the extracts was ascertained using Folin-Ciocalteu's technique. The results were given as milligrammes of the equivalent weight of gallic acid (GAE). It was discovered that the regression coefficient was R2= 0.995. The plot's intercept is 0. 063 and its slope is 0.002. Using gallic acid (20–100µg/ml) as a standard, the total phenolic contents of *N. arbor-tristis* extracts were determined using a regression equation based on a standard curve. It was discovered that the hydroalcoholic extract of *N. arbor-tristis* contained 126.000 mg GAE/gm of total phenolics. Using water as a blank, the absorbance was measured at 510 nm using a UV Spectrophotometer. Standard rutin (20–100 µg/ml) was employed. *N. arbor-tristis* extracts' total flavonoid content was determined using a regression equation based on a standard curve (y=0.0012x+0.092, R2=0.9798). The plot's intercept is zero and its slope is 0.001. It was discovered that the hydroalcoholic extract of *N. arbor-tristis* contained 103.104 mg GAE/gm of flavonoids overall. Figures 1 and 2 in Table 4. One of the easiest and most popular techniques for evaluating the antioxidant activity of natural items is to look for DPPH radical scavenging activity. The method of DPPH radical scavenging was employed to evaluate the antioxidant activity. Ascorbic acid, the control, had antioxidant activity of 25.07µg/ml. The hydroxyl radical, singlet oxygen, and hydrogen peroxide are examples of more reactive species that are formed when superoxide anion is present and cause oxidative damage to proteins, DNA, and lipids. Consequently, one of the most crucial approaches to elucidate the mechanism of antioxidant activity is to investigate the superoxide radical scavenging activity of plant extracts. At a concentration of 1 mg/ml, ascorbic acid, the control, exhibited 36.27 µg/ml of antioxidant activity. The superoxide experiment conducted on hydroalcoholic extracts of *N. arbor-tristis* flower revealed antioxidant activity of 125.23µg/ml at a concentration of 1 mg/ml, as shown in Table 6.8, 6.9, and Figure 6.5, 6.6. Table 5, 6, and Figure 3, 4 show that the hydroalcoholic extract of *N. arbor-tristis* flower yielded 94.28µg/ml antioxidant activity at the same doses. In treated rats, there was no mortality or toxicological indication up to the upper dose of 2000 mg/kg. All 24 rats were healthy throughout the investigation and lived to see the 14-day experiment's conclusion. According to paragraphs 24 and 25 of OECD Guideline 423, animal wellbeing parameters were monitored continuously for the first two hours, then sporadically for up to six hours, and finally daily for up to fourteen days. Every group's experimental observations are methodically documented. Changes in the skin and fur, eyes, mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems, soma to motor activity, and behavioural patterns are among the characteristics taken into consideration. Observations of tremor, convulsion, salivation, diarrhoea, lethargy, sleep, and coma are given particular attention. Animals given an oral hydroalcoholic extract of *N. arbor-tristis* at a dose of 2000 mg/kg did not exhibit any negative effects or death. Table 7. This suggests that the maximum safe dose is 2000 mg/kg. Therefore, 200 and 400 mg/kg of body weight the tenth and fifth, respectively, of the maximum acceptable dose were chosen to be studied for their in vivo anti-hyperlipidaemic effects. The hydroalcoholic extract of *N. arbor-tristis* had an antihyperlipidemic effect on rats fed a high-fat diet; the animals' mean body weight is displayed in Figure 5 and Table 8. Oral HANAT administration (200 mg/kg and 400 mg/kg, p.o.) in the high fat diet induce model significantly decreased serum levels of triglycerides (TG), low density lipoprotein cholesterol (LDL-C), VLDL-cholesterol, and total cholesterol (TC), but significantly increased serum levels of HDL-cholesterol when compared to the positive control group. According to this study, when HANAT was administered to

rats for 30 days at doses of 200 mg/kg and 400 mg/kg, their serum lipid values were significantly ($p<0.001$) lower than those of the control group. When HANAT group animals were given 400 mg/kg, the results were significantly ($p<0.001$) different from the control group. Table 9. The triton-induced study results indicate that, in comparison to the control group, the animals' serum lipid parameters were considerably ($p<0.01$) reduced after receiving HANAT treatment for seven days at dose levels of 200 mg/kg and 400 mg/kg. When compared to the control group, 400 mg/kg of HANAT group animals demonstrated substantial ($p<0.001$) differences. Additionally, an elevated HDL level was noted at this time Table 10.

Table 2: Yield of crude extracts of *N. arbor-tristis* flower extract

Plant	Part used	Method of Extraction	Solvents	% Yield (%W/V)
N. arbor-tristis	Flowers	Soxhlation	Hydroalcoholic solvent (Methanol: Water; 70:30%)	7.8

Table3: Phytochemical screening of extract of *N. arbor-tristis*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Dragendroff's test	+ve
	Hager's test	+ve
	Wagner's reagent test	+ve
2.	**Flavonoids**	
	Lead acetate	-ve
	Alkaline test	+ve
	Shinoda test	-ve
3.	**Tannin and Phenolic compound**	
	Ferric chloride test	+ve
	Lead Acetate test	+ve
	Dilute Iodine solution	+ve
4.	**Glycoside**	
	Borntrager test	+ve
	Legal's test	+ve
	Killer- Killiani test	+ve
5.	**Proteins and amino acids**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Molish's test	+ve
	Barfoed's test	+ve
7.	**Saponins**	
	Foam test	-ve
8.	**Test for Triterpenoids and Steroids**	
	Salwonski Test	+ve
	Libermann and Burchard's test	+ve

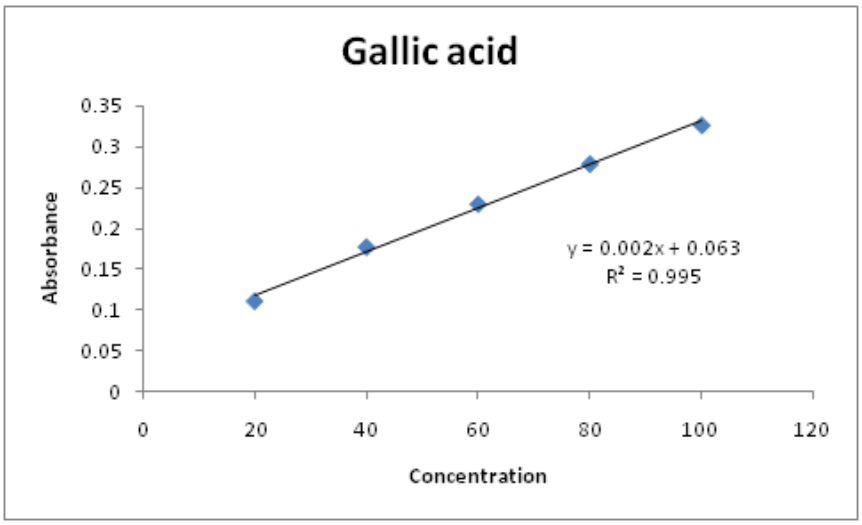

Figure 1: Standard calibration curve of gallic acid for total phenolic content determination

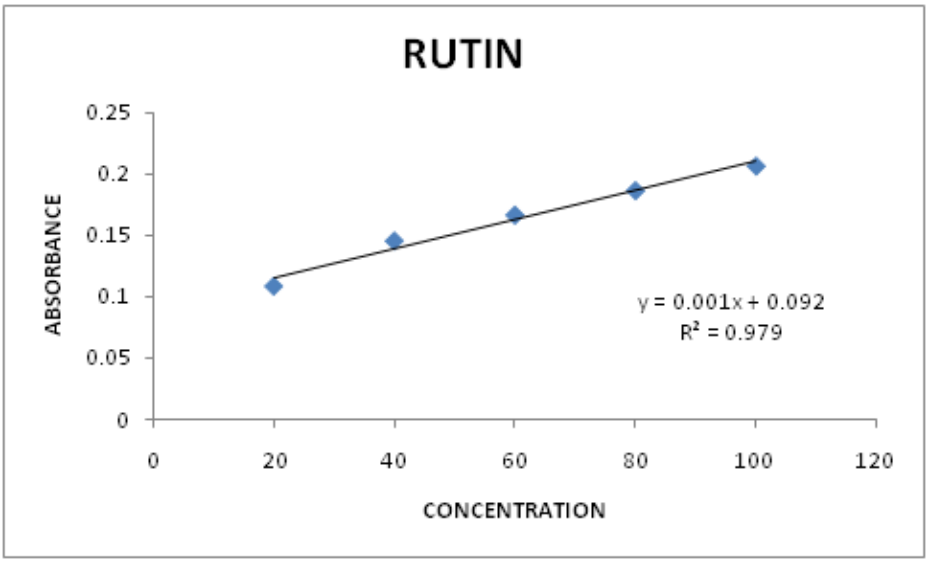

Figure 2: Standard calibration curve of rutin for total flavonoid content

Table 4: Total phenolic and total flavonoid content of *N. arbor-tristis*

S. No.	Extracts	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE)(mg/100mg)
1.	Hydroalcoholic	126.000	103.104

Table 5: DPPH radical scavenging activity of ascorbic acid and hydroalcoholic extract of *N. arbor-tristis*

Concentration (mcg/ml)	% Inhibition ascorbic acid	% Inhibition hydroalcoholic extract of *N. arbor-tristis*
20	48.22	38.26
40	54.98	42.53
60	61.39	45.20
80	69.40	46.80
100	74.38	51.07
IC50 (mcg/ml)	25.07	94.28

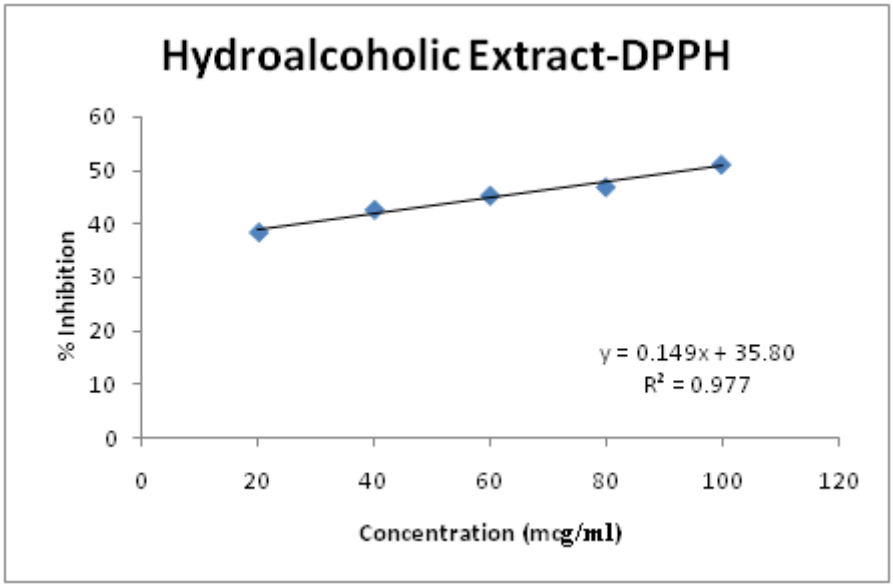

Figure 3: DPPH radical scavenging activity of hydroalcoholic extract of *N. arbor-tristis*

Table 6: Superoxide scavenging activity of ascorbic acid and hydroalcoholic extract of *N. arbor-tristis*

Concentration (mcg/ml)	% Inhibition ascorbic acid	% Inhibition hydroalcoholic extract of *N. arbor-tristis*
20	45.62	31.49
40	52.46	33.26
60	54.53	37.63
80	58.06	41.71
100	60.60	45.31
IC50 (mcg/ml)	36.27	125.23

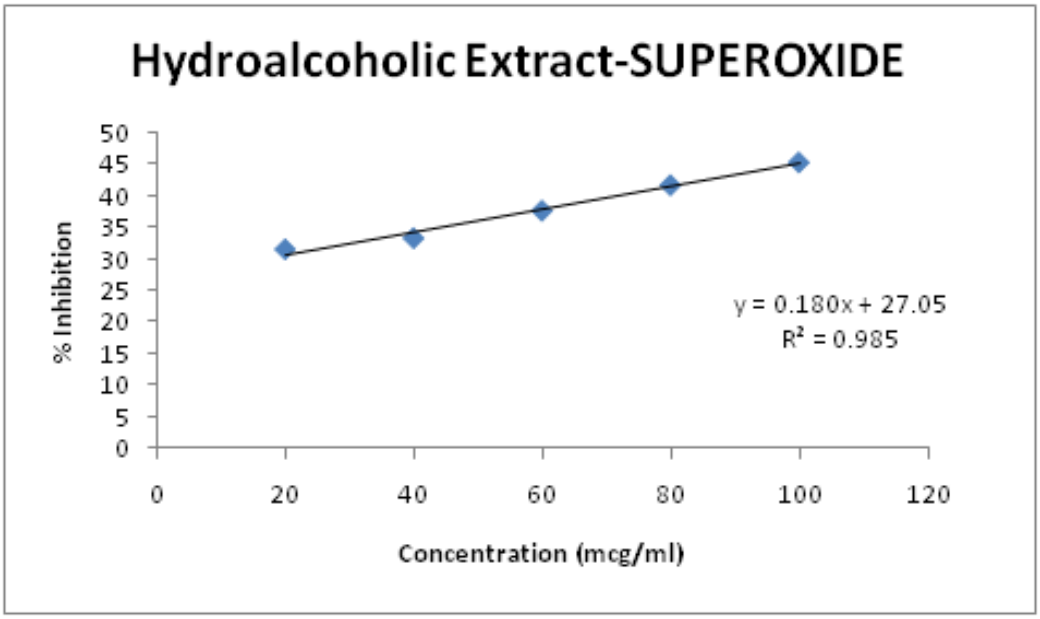

Figure 4: Superoxide scavenging activity of hydroalcoholic extract of *N. arbor-tristis*

Table 7: Change in wellness parameters observed for *N. arbor-tristis* flowers extract treated wistar rats

S. no	Response	Group1(5mg/kg)		Group 2 (50mg/kg)		Group 3 (300mg/kg)		Group 4 (2000mg/kg)	
		Before	After	Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
3	Anxiety	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
4	Roaming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
5	Tremor	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
7	Depression	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
8	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
9	Scratching	Present	Present	Present	Present	Present	Present	Present	Present
10	Defecation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
11	Writhing	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
12	Pupils	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
13	Urination	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
14	Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
15	Skin and fur	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
16	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
17	Piloerection	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
18	Nailstatus	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
19	Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
20	Gauntness	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
21	Lethargy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
22	Diarrhea	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
23	Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
24	Mucous membrane	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Table 8: Effect of HANAT on weight gain in hyperlipidemia-induced rats for 4 weeks

Parameter	Test group	Day0	14thday	28thday
Weight gain	Normal control	126.6±3.10	128±2.36	128.7±2.12
	Positive control	215.4±7.00	228.8±8.21	239.8±7.22
	Atorvastatin 10mg/kg	198.3±4.79***	173.3±5.03***	154±4.55***
	HANAT 200mg/kg	205.5±5.36*	190±7.05***	175.7±5.01***
	HANAT 400mg/kg	193.3±3.78***	176.2±4.68***	167±4.87***

Values are expressed as mean ± SD (n=6).Values were significant when compared with cholesterol group.*P<0.05,**P<0.01,***P<0.001(one way ANOVA followed by Dunnett test), HANAT= Hydroalcoholic extract of *N. arbor-tristis* flowers.

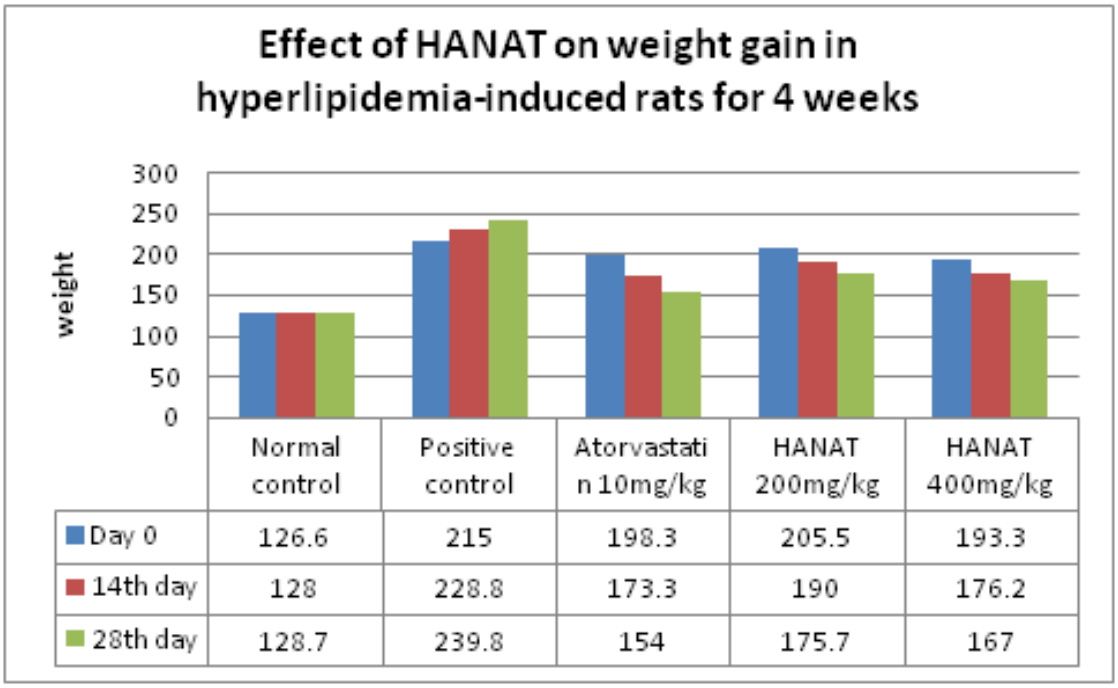

Figure 5: Effect of HANAT on weight gain in hyperlipidemia-induced rats for 4 weeks

Table 9: Effect of HANAT *on* lipid profile in high-cholesterol diet induced hyperlipidemia

GROUP	Total Cholesterol (mg/dl)	HDL(mg/dl)	Triglycerides(mg/dl)	VLDL(mg/dl)	LDL(mg/dl)
Control	84.1±3.78	44.12±1.32	70.89±2.11	14.79±0.41	28.39±3.52
Positive control	148.62±2.5	36.29±2.72	144.15±3.60	27.73±0.65	66.25±2.62
Atorvastatin 10mg/kg	89.82±1.66	48.33±2.02	101.1±2.33***	22.43±0.44	23.13±2.22**
HANAT 200mg/kg	110.32±2.34	42.81±1.90	118.02±2.10**	25.5±0.32	46.53±2.23
HANAT 400mg/kg	94.35±1.73**	45.49±2.17***	103.06±2.87	22.6±0.50	30.59±1.97**

Values were mean ±sd (n=6). Values are statistically significant at *P<0.05 and more significant at **P<0.01,***P<0.001 Vs hyperlipidemic control using one way ANOVA followed by Dunnet's test, HANAT= Hydroalcoholic extract of *N. arbor-tristis* flowers.

Table 10: Effect of HANAT on lipid profile in Triton induced hyperlipidemia

GROUP	Total Cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Control	84.43±0.20	44.70±0.68	69.22±0.77	16.04±0.49	28.08±0.85
Positive control	147.15±2.23	33.04±2.33	129.54±2.54	29.31±0.97	68.98±1.93
Standard (Atorvastatin 10mg/kg)	88.39±0.91***	47.40±0.70	78.07±0.53	20.75±0.34**	24.74±0.59
HANAT (200mg/kg)	110.56±0.94	41.12±0.52	98.15±0.62*	25.88±0.5	40.35±0.61
HANAT (400mg/kg)	93.36±1.71***	44.79±0.90**	84.18±0.86	22.5±0.94	27.09±0.73**

Values were mean±sd (n=6). Value are statistically significant at *P<0.05andmoresignificantat**P<0.01, ***P<0.001Vs hyperlipidemic control using one way ANOVA followed by Dunnet's test, HANAT= Hydroalcoholic extract of *N. arbor-tristis* flowers.

DISCUSSIONS

Hyperlipidemia is the term used to describe a high cholesterol state that has been shown to raise total cholesterol, raise the risk of atherosclerosis, and ultimately result in cardiovascular disease. Herbs are essential for the treatment of many CVDs. In India's traditional medical system and ethnomedical practices, a variety of medicinal plants and their preparations are utilised to treat hyperlipidaemia. We do not, however, have a sufficient treatment for hyperlipidaemia; instead, the majority of herbal medications expedite the lowering of cholesterol through a balanced diet. Thus, the quest for rats with elevated

cholesterol levels that have anti-hyperlipidaemic activity. *N. arbor-tristis*, a big shrub in the Oleaceae family, is often referred to as Night Jasmine or Parijata[42]. It originated in India and is now found throughout Bangladesh, the Indo-Pak subcontinent, South East Asia, and both tropical and sub-tropical regions of the region. *N. arbor-tristis* is a revered, attractive, and medicinally significant shrub that is well-known throughout the nation for its fragrant white blooms. Nearly every component of this plant, including the roots, bark, leaves, flowers, seeds, and seed oil, has medicinal and industrial value. Given that it is regarded as one of the five wish-granting trees of Devaloka in Hindu mythology, garlands of flowers are produced as sacrifices to the gods. The textile industry uses orange dye made from flowers to colour cotton and silk[43].7.8% w/v was obtained using the soxhlet extraction process for 250 grammes of powder. Alkaloids, sugars, glycosides, tannins, phenolic chemicals, triterpenoids, and steroids are found in plant flower extracts, which may account for their antioxidant and anti-hyperlipidemic capabilities. The specific pharmacological effects were caused by the presence of several phyto-constituents such as triterpenoids, alkaloids, glucose, glycosides, tannins, and phenolic compounds. The amount of phytoconstituents is displayed in hydroalcoholic extracts. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) assays were used to quantitatively screen crude extracts for phytochemicals by confirming the presence of flavonoids and phenolics in plant material. The measurement of the overall phenolic content, represented in milligrammes per gramme of gallic acid equivalent (GAE). It was discovered that the hydroalcoholic extract of *N. arbor-tristis* contained 126.000 mg GAE/gm extract of total phenolics. The extracts' overall flavonoid concentration was given as mg rutin/gm dry weight (mg rutin/gm DW). The hydroalcoholic extract of *N. arbor-tristis* was found to have 103.104 mg RE/gm of total flavonoids. Chemicals known as antioxidants, also known as free radical scavengers, interact with and neutralise free radicals to stop them from damaging biological systems' cells[44,45]. The effectiveness of natural antioxidants as pure chemicals or as extracts from plants has been measured using in vitro techniques for assessing antioxidant activity. The results of the antioxidant screening indicate that DPPH radicals were reduced in power. The hydroalcoholic extract of *N. arbor-tristis* was shown to have an inhibitory concentration 50% (IC50) value of 94.28µg/ml under DPPH radical scavenging activity, which was higher than that of ascorbic acid, which was determined to be 25.07µg/ml. Regarding concentration, an activity that was dosage dependant was noted. When comparing the hydroalcoholic extract to the conventional medicine, the percentage of inhibition is lower when taking into account research on super oxide radical scavenging. Acute phase toxicological investigations on treated rats show no mortality or toxicity indicators up to a dosage limit of 2000 mg/kg. All 24 rats were healthy throughout the investigation and lived to see the 14-day experiment's conclusion. When *N. arbor-tristis* hydroalcoholic extract (2000 mg/kg) was given orally to animals, no negative effects or death were noted. This suggests that the maximum safe dose is 2000 mg/kg. Therefore, 200 and 400 mg/kg of body weight, or 1/10th and 1/5th of the maximal safe dose, were chosen to investigate the antihyperlipidemic activity in vivo. In this investigation, rats were given a high-fat diet as part of an experimental induction procedure. Cholesterol accumulates in endothelial cells and is transported into the bloodstream by lipoproteins. This causes the body's levels of TC, TG, LDL, and VLDL to rise and HDL to fall. It is produced in the liver, where it is changed into bile acids and eliminated via the face. Hypercholesterolemia can lead to oxidative stress, which can lead to urinary failure. By assessing bodyweight and the activities of different lipid profiles, such as TC, TG, HDL, LDL, and VLDL, one may evaluate the function of cholesterol. When rats with hyperlipidaemia are fed a high-cholesterol diet, their body weight may rise. Increased lipoproteins that are absorbed from the intestine and processed in the liver can be transported into various tissues as dietary fat, or FFAs[46]. By lowering LDL and VLDL cholesterol levels and raising HDL levels, the HANAT therapy and the medication atorvastatin dramatically reduce body weight. When hydroalcoholic flower extracts were given orally to animals that had been forced to become hyperlipidaemic due to a high cholesterol diet, the results showed a large decrease in cholesterol, triglycerides, low density lipoproteins, and very low-density lipoproteins, as well as a significant increase in HDL cholesterol. The p value ($p<0.001$) indicated that the results were significant. The non-ionic detergent Triton WR 1339 causes acute hyperlipaemia by increasing cholesterol levels two to three times in a 24-hour period after treatment. Because Triton can obstruct the tissue's ability to absorb plasma lipids, it is believed that this causes increased hepatic synthesis of cholesterol, which is the mechanism underlying Triton-induced hypercholesterolemia. The triton-induced study results indicate that animals treated with HANAT at dose levels of 200 mg/kg and 400 mg/kg had considerably lower blood lipid parameters ($p<0.01$) than the control group; however, the 400 mg/kg of HANAT group animals showed significantly higher serum lipid parameters ($p<0.001$) than the control group. Additionally, a higher HDL level was noted at this time.

CONCLUSION

Antihyperlipidemic activity and possible antioxidant activity overall were revealed by the experimental and research findings pertaining to the full study of *N. arbor-tristis* flowers. Numerous antioxidant in vitro experiments conducted on the *N. arbor-tristis* flower extract show that its radical scavenging ability is well-established and roughly equivalent to that of ascorbic acid. Studies conducted on animals using hydroalcoholic extracts of *N. arbor-tristis* flowers showed that the extracts had a highly promising antihyperlipidemic effect, as evidenced by the considerable reduction in lipid levels observed in every animal group administered with the extracts. As a result, the animal group that received a higher extract will exhibit more activity than the group that received a lower extract or the conventional antihyperglycemic medication. Therefore, it can be concluded that the potential advantages of *N. arbor-tristis* hydroalcoholic extracts have been well-established and can be further utilised to demonstrate the antioxidant and antihyperlipidemic activity for regulating lipid levels and lowering the likelihood of ROS generation against free radicals. According to the research's previously reported findings, *N. arbor-tristis* may offer antioxidant and antihyperlipidemic properties. It is therefore advised that more research be done in order to determine the precise mechanism underlying the action of hyperlipidemia.

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