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Original Research Article

Augmented Anti-Hyperlipidemic Efficacy of Dolichos Aciphyllus Through Nanotechnological Amplification

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Abstract: ***Background***: According to the past studies, the beans of herbal plant Dolichos aciphyllus (DA) was used in the treatment of several ailment-like cardiovascular diseases, diabetes etc. The primary causes of coronary heart disease can be hypolipidemia. The plant Dolichos aciphyllus used in the treat hyperlipidemia with lesser adverse drug reactions. ***Materials and Methods***: The current research aims to test out the efficacy of the ethanolic extract of Dolichos aciphyllus and its Nanoparticle's anti-hyperlipidemic activity in HFD model in the Hyperlipidemic rats. The end point parameters were evaluated for anti-Hyperlipidemic activity is total cholesterol, triglycerides, HDL, LDL, liver enzymes. The anti-oxidant activities were performed to analyze the effectiveness of DA to prevent the oxidation of molecules by scavenging free molecules. ***Results***: In the recent investigation it is significantly proved that the high dose of DA and DA NP has anti-Hyperlipidemic activity as it showed considerable reduction in the levels of serum of biochemical parameters like significant decrease in total cholesterol, TG, LDL, VLDL, HDL, the levels of AST AND ALT when compared to Atorvastatin. The stage of MDA and GSH proved antioxidant activity of the plant extract. The outcomes of histopathological reading proved the liver cells of the hyperlipidemia induced group was damaged which was recovered in the DA extract and DA NP treated group. ***Conclusion***: The beans of Dolichos aciphyllus extract and its formulation was proved to be a potential anti-Hyperlipidemia agent in addition to the current and existing Hyperlipidemia treatment.

Keywords: Hyperlipidemia, HMG CoA Reductase, High Fat Diet, Quercetin, Flavonoids, Atorvastatin.

INTRODUCTION

Hyperlipidemia may be caused by inappropriate amount of intake of lipid which can be described by the increased lipids amount in serum like total cholesterol, low density lipoprotein (LDL), triglycerides (1). Hyperlipidemia is the death causing factor due to various CVS diseases, coronary disease and atherosclerosis. The endothelial dysfunction which is the major reason of atherosclerosis enhances the LDL permeation through the layers which causes atherosclerotic damage (2, 3). The enzymatic and non- enzymatic antioxidant mechanism of the cadaver is responsible to control the discrepancy of lipid caused through preventing the effect of oxidation, hindering the lipid peroxidation, scavenging of free radicals and sustaining the redox balance in the cells(4). There are several advantageous antioxidants found in the exogenous sources other than endogenous antioxidants. The antioxidant potential is found in various phytochemical constituents of plants like phenolic compounds, flavonoids, anthocyanins, terpenes, catechins etc (5). Due to the availability of ample amounts of various antioxidants in the plants, they are the most preferable substitute for the formulation of novel entity (6) for treating several diseases like increase level of cholesterol in blood, ulcer, enhances detoxification of blood and sarcoma (7).

The beans of Dolichos aciphyllus (DA) which is a subtropical plant has a family of Fabaceae employed to treat several disorders like anthelmintic, expectorant, diuretic, ophthalmic and also used as a tonic. The seeds have been found with several pharmacological activities like antilithiatic, anti-hepatotoxic and anti-oxidant activity (8, 9, and 10). The sub erect and branched herb has also been used in treating obesity, hepatomegaly, splenomegaly and

renal calculi. The Phytochemical constituent Quercetin which is a flavonoid present in the species of Dolichos aciphyllus makes it potent to treat CVS and other diseases. So, the existent intend of the research swot up is to analyse the efficacy of Dolichos aciphyllus seeds to reduce the intensity of lipids in the serum on female Wistar Albino rats induced by High fat Diet thereby proving the subtropical plant as a traditional means of healing Hyperlipidemia as there was no previous work reported with this specific species of Dolichos. The herbal drug extract formulation can be a promising therapy to treat the biochemical parameters of Hyperlipidemia along with the existing synthetic treatment in the market with lesser adverse drug reactions and more efficacies (11).

MATERIALS AND METHODS:

Chemicals: the herbal extract of Dolichos aciphyllus has been purchased from Ambe NS Agro Product PVT. LTD. Vasundhara, Ghaziabad, UP. Other chemicals like Chloroform, Formaldehyde, Glacial Acetic Acid, Potassium Dihydrogen Orthophosphate, Sodium Chloride, Sodium Carbonate, Sodium Bicarbonate, Sodium Tartarate, Thiobarbituric Acid, Ellman's Reagent, Bovine Albumin Fraction, Folin And Ciocalteu Phenol Reagent has been purchased from Central Drug House, Ansari Rd, Daryaganj, Delhi. Other chemicals have been obtained of analytical grade from Merck LTD India.

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2.1 Phytochemical Screening and Fingerprinting Analysis:

The initial phytochemical components analysis like alkaloids, flavonoids, tannins, saponins, phenols, triterpenoids, carbohydrates, proteins has been performed with the extract of Dolichos aciphyllus. The tests have been performed according to the standard procedures (12.13).

The Finger Printing analysis for the DA extract and standard Quercetin present in the extract was done through HPLC Technique attached with Shimadzu U.V detector. A 10 ml amount of the DA extract and standard was calculated and filtered using a 0.45 µm mesh size membrane filter followed by 10 minutes

sonication and both the samples were filtered using 0.45µm size membrane filter. The filtrate was kept in vials measuring 2.5ml and injected in the HPLC auto sampler in triplicates. The Phytochemical constituents were separated in a C18 column with a 0.8 ml/min rate of speed. The elution solvent used as water & acetonitrile in a ratio of 70:30 with 0.5% v/v

phosphoric acid for 30 minutes at 356 nm wavelength(14).

Physicochemical Parameters

2.2.1 Content of Heavy Metals in DA Extract:

The presence of amount of heavy metals like lead, calcium, cadmium, copper, iron, magnesium, nickel, zinc in the DA extract was calculated by an atomic absorption Spectrophotometer auto sampler. The content of heavy metals was calculated by taking 1 ml of the concentrate and muddled up with nitric acid, concentrated HCL and water in a ratio of 3:1:1 and transferred to a 250 ml beaker followed by heating at 150°c till a homogeneous mixture was attained. The final amount was adjusted up to with 50 ml pure water and the specimens were investigated with atomic absorption spectroscopy in duplicates (15).

2.2.2. Analysis of Microbial Quality in DA Extract

The microbial load evaluation of the herbal extract DA was determined to ensure high quality and safety. For the feasible bacterial count MacConkey agar was used while for moulds and yeast potato dextrose agar was employed. Cetrimide Nutrient agar & Bismuth sulphite agar were utilized. For selective isolation of Pseudomonas and Salmonella species Cetrimide Nutrient agar & Bismuth sulphite agar were availed respectively. According to the given directions on the package all Medias were prepared. To uniformly distribute the microbial content, the herbal extract was shaken dynamically. The stock solution was composed by thinning 1 ml of plant extract with 9 ml of sterile demineralized water under aseptic condition followed by serial dilutions to achieve a concentration of 106. 1 mL of the dilute test sample was inoculated in McConkey agar plate by spread plate method and incubated at 37 ◦C for 48 h for fixing viable bacteria number. 1 ml test sample was streaked on plate containing potato dextrose agar media and placed in incubator at 25 ◦C for five days for the total fungal count. The results were represented in duplicates. The colonies were counted using colony counter. The load of micro- organism was calculated as colony forming units per mL (CFU/ mL (16)

2.2.3 Entire Content of Flavonoid:

The method of AlCl3was utilized to calculate the entire matter of flavonoid present in Dolichos aciphyllus extract (17).500 mg drug and 20 ml methanol were mixed and kept for 72 hours in a shaker incubator with continuous agitating and later on filtrating through Whatman filter paper. The remainder was converted into a semi-solid residue by concentrating at a temperature below 40°c which was dissolved again in methanol to form a solution of 1000 ppm. From the stock solution, a mixture of 0.5 ml was withdrawn and blended with 1.5 ml methanol, 0.1ml 10% AlCl3 solution, 0.1 ml 1 M potassium salt of acetic acid solution , 2.8 ml distilled water respectively. Absorbance of the above mixture was taken at 370 nm. Quercetin was taken as reference standard for calculating total flavonoid content and articulated as Quercetin equivalent/100gm for the extract.

2.2.4 Entire content of Phenols:

Total phenol content in the extract of Dolichos aciphyllus was analyzed by using the Folin-ciocalteau method (18). Initially, 500 mg drug extract and 10 ml 80% ethanol were homogenized and centrifugation at 10000 rotation per minute until 20 minutes. The liquid above dregs was withdrawn after being re extricated using 5 ml of ethanol followed by evaporation. The above dry residue was solubilised by using 10 ml of pure water. From above stock solution, 0.1 ml of aliquots was withdrawn and the finishing amount was amended with 3 ml distilled water and 0.5 ml 1 N Folin-Ciocalteau reagent and 2 ml of 20% Sodium Carbonate was added to the above mixture after 3 minutes. Obtained mixture was heated for 1 minute on water bath, cooled. Absorbance was quantified at 254 nano meters. Gallic acid was used as a paradigm for calculating total phenolic compound and communicated as gallic acid equivalent/100 gm of the extract.

2.2.4 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The extract of Dolichos aciphyllus was evaluated for antioxidant activity using the procedure of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity (19). Ascorbic acid was employed as reference calibre to evaluate the antioxidant activity of concentrate with different concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml in methanol along with same strength of plant extract.

0.1 mM DPPH was put in order in methanol & 2.4ml of this solution has been added in each dilution of both the solutions. This reaction mixture was mixed well with the help of vortexed and kept aside for thirty minutes. 0.1mM solution of DPPH in methanol was used as blank. After 30 minutes the absorbance was quantified by U.V spectrophotometer at 517 nano-meters. All above procedure has been performed in triplicate. Percentage inhibition of standard and extracts were calculated by:

I %: (Ac - At)*100 / Ac
I% = Percentage Inhibition Ac = Control absorbance
At = Standard absorbance / Plant extract absorbance

2.3. In-vitro Studies

2.3.1 Inhibition of pancreatic lipase action

The Hypolipidemic efficacy of the different concentrations of DA concentrate and the prepared formulation in ethanol and ethyl acetate was analyzed by the pancreatic lipase inhibition assay method. The different concentrations of DA extract and the

prepared formulation at same concentrations were incubated in a combination mixture of 1 ml of single unit of lipase enzyme and 7.2 pH 100ml phosphate buffer followed by addition of 0.5% of Triton- X-100. Addition of p-nitrophenyl butyrate stimulates the enzyme activity which was observed at 340 nm (20).

2.3.2. Inhibition of Malic Dehydrogenase action

The reduction of NADP at 340 nm was utilized to analyze Malic dehydrogenase activity. The different concentrations of DA extract and the prepared formulation at same concentrations were incubated in a mixture of 1 ml of Tris-Hydrochloric Acid at pH 7.4, 0.5 unit of malic dehydrogenase enzyme, L-malate and manganese (II) Chloride. Addition of 2mM NADP stimulated the enzyme reaction which was regulated at 340nm (21).

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In-vitro Hypolipidemic function of both ethanol and ethyl acetate Extract DA and formulation were analyzed. The reduction in assimilation of NADPH to NADP was quantified at 340nm followed by the

estimation of HMG-CoA Reductase enzyme action. Based over highest % inhibitory outcome of HMG-CoA Reductase activity of the extracts as well as the formulation was used for the further assessment of In-vivo hyperlipidemic activity in the HFD rats (22).

2.4. Formulation of DA Extract Nano-lipid Carriers (DA NP):

Dolichos aciphyllus loaded Nano-lipid carriers (DA NP) was prepared with the help of homogenization method and ultrasonication by using Stearic acid as solid lipid, Oleic acid as liquid lipid and Tween 80 as surfactant. Solid lipid and liquid lipid were melted together at same temperature to form the lipid phase and the measured amount of drug(DA extract) was added to the melted lipid solution with regular stirring till a clear solution was obtained followed by the addition of preheated Tween 80 aqueous solution as the liquid phase. The lipid phase and the aqueous phase mixture form the pre-emulsion. The prepared hot preheated pre- emulsion was kept under high speed homogenization at 200 rpm for 30 minutes and ultrasonication for 6 minutes with on-off method which prepares the nano-emulsion. On cooling the nano-emulsion Nano-lipid carriers were formed by lipid phase re-crystallization (23)

2.5. Animals:

Adult female Wistar Albino Rats weighing 100-150 grams were purchased from Central Animal Facility, AIIMS, New Delhi. After purchasing the animals were breaded 3 in groups in each cage in an AC area at 23 ± 2°c temp and 55 ± 5% humidity which was provided with light/dark cycle. The rats were given with a diet of water and libitum for 1 week. The experiments on all the albino rats were performed according to Institutional Review Board of Organization (CPCSEA Number ITS/02//IAEC/2022). Animals were provided with High Fat Diet (HFD) containing 61% fat, 21% protein & 21% carbohydrate (24). Control group was given standard commercial diet including 12.5% fat, 22.8% protein and 68.7% carbohydrate. Just about 48 Wistar albino rats were selected for the anti-hyperlipidemic activity which was induced through HFD till time duration of 90 days. Animals which weighed near about 230-250 g have been chosen for the further studies (25). The remaining group of rats were given with normal pallet chow diet and considered for the control group.

2.6. Acute Oral Toxicity:

Acute oral toxicity was performed for the methanolic concentrate of Dolichos aciphyllus according to OECD guidelines. Wistar albino rats were chosen randomly for the test and kept individually in polypropylene cages maintaining standard condition i.e; 12 hour light and dark cycle and 30-60% humidity. The animals were given normal pellet diet and water. Before starting the dosing for the test, the animals were kept fasted. The sample test was given in a distinct dose to a set of 5 animals with a single oral dose of 1000 -8000 mg/kg using feeding tube which increases the abnormalities in behaviour in the rats followed by increase in mortality rate with the elevation in the dosage. During the test period the animals was noticed for the first 30 minutes then for 24 hours at regular intervals. No mortality and no change in body weight in the rats were noticed with a dose of 1000, 2000 and 4000 mg/kg during the period of test. According to OECD guidelines the dose for further in-vivo study was selected as 1/10th of the dose used for the acute oral toxicity. (26)

2.7. Experimental Design:

54 wistar albino rats were used weighing 100-150 grams. The rats have been segregated in 9 groups (n=6 each group). The experimental design has been represented in table: 1

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Table no: 1 Experimental Design

Number	Details of Group	Induction of	Dose of Drug	Total

of Groups		Disease &Regimen		number of Animals
G1	Control Group	NA	0.9 % Nacl	6
G2	Negative Control	HFD	NA	6
G3	Positive Control	HFD + Atorvastatin	10 mg/kg	6
G4	T1-Extract-1	High fat diet+ *Dolichos aciphyllus* (DA)extract	100 mg/kg	6
G5	T2-Extract-2	High fat diet +*Dolichos aciphyllus* (DA)extract	200 mg/kg	6
G6	T2-Extract-3	High fat diet +*Dolichos aciphyllus* (DA)extract	400 mg/kg	6
G7	T1-Extract Loaded formulation 1	High fat diet +*Dolichos aciphyllus* (DA)extract Loaded Formulation	100 mg/kg	6
G8	T2- DA Extract Loaded formulation 2	High fat diet +*Dolichos aciphyllus* (DA)extract Loaded Formulation	200 mg/kg	6
G9	T3- DA Extract Loaded formulation 3	High fat diet +*Dolichos aciphyllus* (DA)extract Loaded Formulation	400 mg/kg	6

The dose for the extract Dolichos aciphyllus has been decided on the basis of acute oral toxicity studies. The study proved that extract of Dolichos aciphyllus did not indicate any toxicity or mortality with the dose of 4000 mg/kg. So, the doses have been chosen about 1/10th and 1/5th of 4000 mg/kg which is 200 mg/kg and 400 mg/kg body weight (27). All the doses of the DA extracts and the formulation along with HFD have been given for one time in a day for duration of 90 days (28, 29). The rats have been sacrificed after completing the experimental study period through cervical dislocation. Biochemical analysis has been by collecting the blood serum sample through segregating liquids of dissimilar densities at 3000 rpm at 4°c for 20 minutes. Liver tissue has been separated, cleaned with 0.9% ultra chilled salty solution, dried towel which has been applied for other studies. The one half has been used to measure MDA and GSH after storing at -20°c and the other half has been fixed for 24 hours in 10% buffered formaldehyde for further histopathological examination.

Measurement of physical parameters

Estimation of body weight change:

The body weight of the individual animals has been taken on daily basis from the start of work till the animal died. Alters in the B.W has been recorded.

Measurement of Average food intake

Food intake has been estimated on daily basis by isolating the spilled food while the animal was consuming the food to obtain an accurate consumption of food intake (30).

Other Biochemical Analysis:

Other biochemical estimations such as i.Cholesterol (31) ii.Triglycerides (32) iv.HDL vi.MDA (33) vii.GSH (34) viii. SOD (35) ix.Catalase (36), x. AST (37), xi. ALT (38) were been performed by using standard procedures. VLDL and LDL have been calculated by using the subsequent formula (39)

VLDL = Total Serum TG/5 LDL= {TC – HDL-TG/5}

Histopathological Studies:

For histopathological studies, a section of liver has been sliced for the different groups and stored in a well closed container in 10% formalin for 12 hours (40). The results have been acquired and checked for histopathological changes in the normal, damaged and recovered liver.

Statistical Analysis:

The acquired data has been subjected to statistical analysis and the results have been articulated as ± Standard Error of Mean (S.E.M). The significant differences in between the means of various groups has been concluded on the basis of One-way analysis of variance (ANOVA) followed by Duncan's multiple test range. A P value of ≤ 0.05 has been considered as significant.

RESULTS:

Testing of Phytochemical Components:
The data of the phytochemical analysis has been presented in table: 2

Table No: 2 Analysis of Phytochemical Components of Ethanolic Extract of Dolichos aciphyllus

SLNO	Phytochemical Constituents	Ethanol	Ethyl Acetate
I.	Steroids	-	-
II.	Terpenoids	-	-
III.	Tannins	+	+
IV.	Flavonoids	+	+
V.	Proteins	-	-
VI.	Glycosides	+	+
VII.	Phenolic Compounds	+	+
VIII.	Saponins	-	+
IX.	Alkaloids	+	+
X.	Sugars	+	+

+) indicates presence (-) indicates absence of that chemical constituent in the sample.

3.1.2 Finger Printing Analysis:
The finger printing analysis for Dolichos aciphyllus extract and the quercetin was done through HPLC. The data was represented in fig: 1

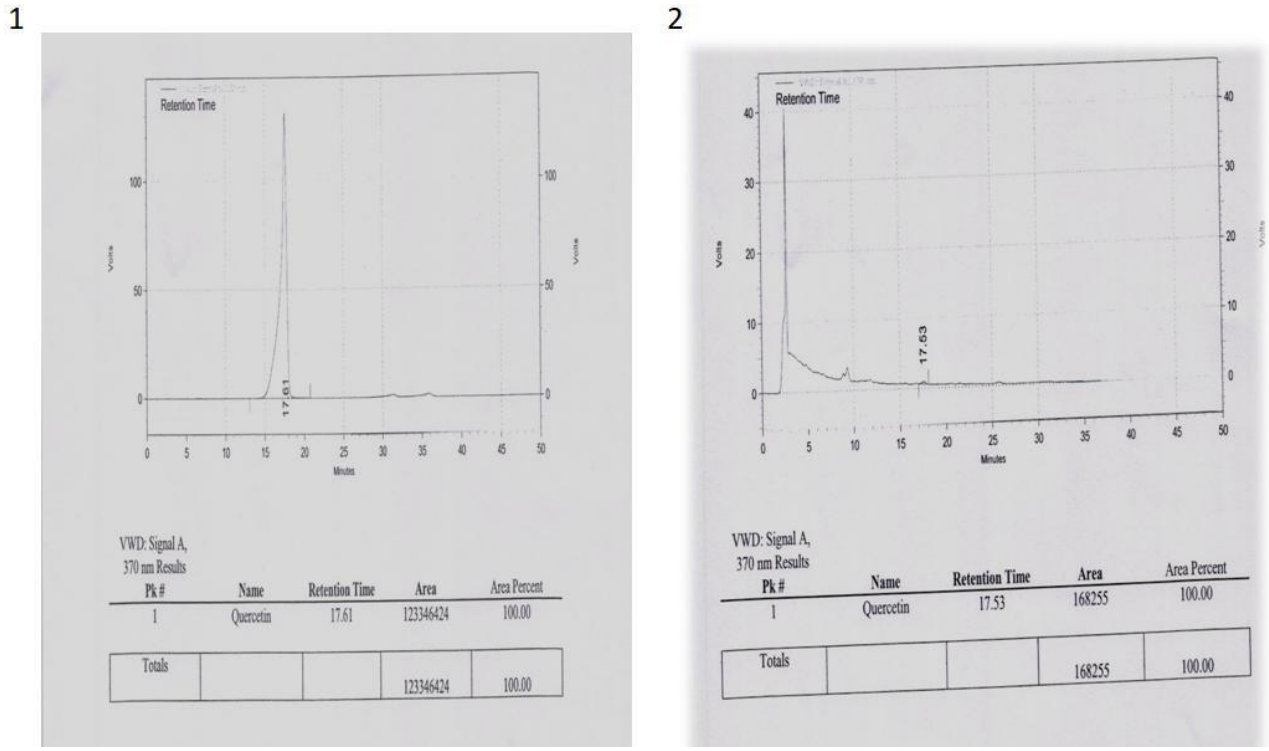

Fig No1: (1) HPLC Chromatogram of standard Quercetin

(2) HPLC Chromatogram of Dolichos aciphyllus

Physicochemical Parameters:
Content of Heavy Metals in DA Extract:
The heavy metal concentration in two groups of the DA extract was estimated. No significant difference ($p > 0.05$) in the content of heavy Metals. The results were monitored through two tailed unpaired t-test and symbolized in table: 3

Table no: 3 Content of Heavy Metals

Elements	Concentration in DA Extract

Lead	28.3 ± 1.99
Calcium	98.44 ±0.53
Cadnium	0.0035±0.0012
Copper	2.838 ± 0.0538
Iron	24.91 ± 0.672
Zinc	15.29 ± 0.402
Nickel	5.91 ± 0.626

Results are means ± sd (N = 2).

3.2.2. Analysis of Microbial Quality in DA Extract

The total microbial count in 2 batches of DA extract such as Ecoli, salmonella, P aeroginosa and S. aureus were absent where as yeasts and moulds compiled within the acceptable limits. There was no significant difference (p> 0.05) within two batches in micro-organisms content. The results were represented in the table no: 4

Table no:4 Microbial content

Microbes	Microbiological analysis (cfu/gm)	
	BI	B2
E.Coli	Absent	Absent
Pseudomonas aeruginosa	Absent	Absent
Salmonella	Absent	Absent
Staphylococcus aureus	Absent	Absent
Yeast & Mould Counts	NMT 100 Cfu/Gm	NMT 100 cfu/gm

Total Flavonoid & Phenolic Content

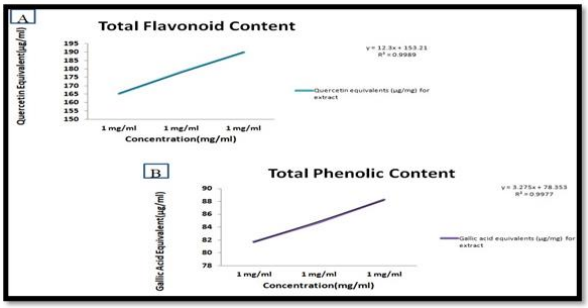

Fig no: 2 (A) Quercetin equivalents (µg/mg) for Dolichos aciphyllus for total flavonoid
(B) Gallic Acid equivalents (µg/mg) for Dolichos aciphyllus for total Phenolic Content

3.2.4. Antioxidant Analysis of DA extracts

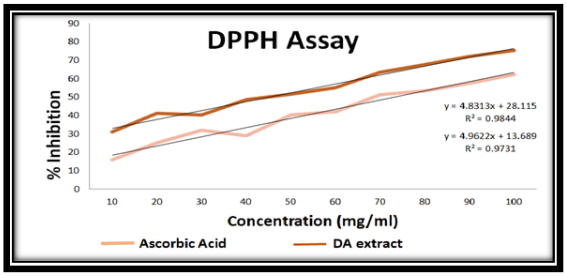

Fig no: 3 IC50 Calculation and Antioxidant analysis by DPPH method

In-vitro antioxidant activity

The entire flavonoid content in DA extract was detected 187.88 mg of Quercetin equivalent/gm of the drug extract while the total phenolic content in the DA concentrate was detected 87.72 mg of Gallic acid equivalent/gm of the drug extract. The reducing power of the DA extract has been augmented with the raising concentration followed by highest activity. Acquired results of DPPH with IC50 have been found to be 40.61µg/ml. The standard ascorbic acid

has been taken as reference which also indicated same IC50 value. The results have been represented in fig: 2 & 3.

Inhibitory Concentration of DA extract:

The DA concentrate of ethanol was established to be more efficacious on Pancreatic lipase and Malic dehydrogenase enzyme which helps in reducing synthesis of lipids in the body. The data has been represented in fig : 4

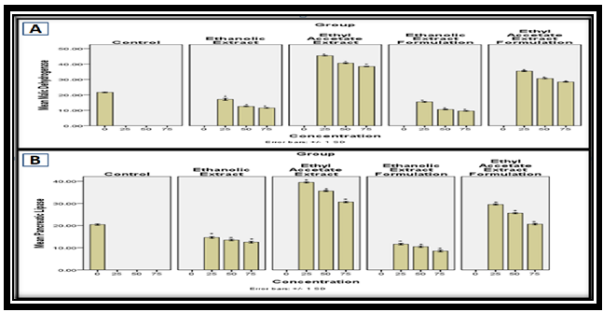

Fig No: 4 Inhibitory concentrations of DA Extract at 25-75µm concentrations. Statistically significant values are expressed as *p<0.05. The result expressed as mean ± SD (n=3). The statistical difference between the DA treated group, control group, Standard group (Atorvastatin) was analyzed by one way ANOVA followed by Dunnet test.

In-vitro Hypolipidemic study

HMG-CoA Reductase, the primary enzyme in the anabolism of cholesterol. The acquired data indicates the in-vitro Hypolipidemic efficacy of DA Extract According to results DA concentrate of ethanol and DA NP reduced the enzyme activity when compared to the control group as in fig: 5. According to the obtained data Ethanolic extract of DA & DA NP were having maximum inhibition (77.12% and 86.81%) when compared to other extract and standard drug Atorvastatin (90.56%) which was represented in table: 5 . As a result, the extract with highest % inhibition and enzyme activity was subjected to further in-vivo hyperlipidemic activity in HFD Rats.

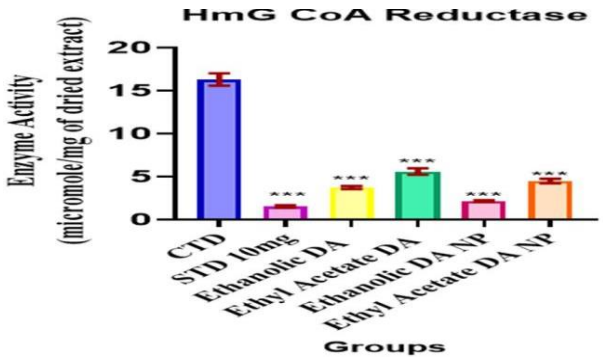

Fig No: 5 In vitro Hypolipidemic effect of DA Extract on HMG-CoA Reductase. The result expressed as mean ± SD (n=3). The statistical difference between the DA treated group, control group , Standard group (Atorvastatin)was analyzed by one way ANOVA followed by Dunnet test. *** p 0.001 vs control group

Table No: 5 % inhibition DA Extract and DA Extract Formulation

Sample	% Inhibition
Control	
Atorvastatin10mg	90.5667
DA Ethanolic Extract	77.1267
DA Ethyl Acetate Extract	65.6333
DA Ethanolic Extract NP	86.8167
DA Ethyl Acetate Extract NP	72.2667

% of inhibition was calculated by using the formula Control value- test value x 100 / control value

Acute Oral Toxicity

After administering 8000 mg/kg dose of DA Extract orally the rats became less active and weight loss was observed. Based on the findings, the acute oral toxicity results specified that extract of Dolichos aciphyllus was found safe with dose of 4000 mg/kg b.w with nil mortality after 14 days of treatment followed by no abnormalities. So, the oral LD50 for DA extract was selected as 100mg/kg, 200 mg/kg, 400 mg/kg b.w for the further In-vivo study according to OECD Guidelines

Analysis of Physical Parameters

3.7.1 Effect of DA extract and DA NP on Body Weight and Food Intake

The first reading of the experimental animals was taken at experiment's 1st day followed with alteration in their b.w has been initially recorded at an duration of 15 days after the treatment given to the experimental rats through DA extract followed by 30 days. The weight of the animal of the group given with HFD has been notably ($p < 0.05$) amplified indicating effect of diet given to the group of animals. The increased weight was significantly ($p< 0.05$) reduced after administering DA extract particles along with atorvastatin (10mg/kg) after assessing against HFD group of rats. The group of rats was given with DA Extract and DA NP in high dose significantly reduces the B.W. The data clearly designates towards the diminution of b.w in treated group animals in dose dependent manner (100mg/kg, 200 mg/kg and 400 mg/kg b.w) of DA concentrate & DA NP.

The first reading of the experimental animals was taken at experiment's 1st day followed with alteration in their b.w was initially recorded at an duration of 15 days after the treatment given to the experimental rats through DA extract and DA NP followed by 30 days and on the basis of the acquired data it was brought to conclusion that the day by day intake of food was slightly reduced in the DA treated group of animals & DA NP (100mg/kg, 200 mg/kg and 400 mg/kg body weight) after evaluating the animals fed through HFD diet alone. The results of body weight and Food Intake were graphically represented in fig: 6

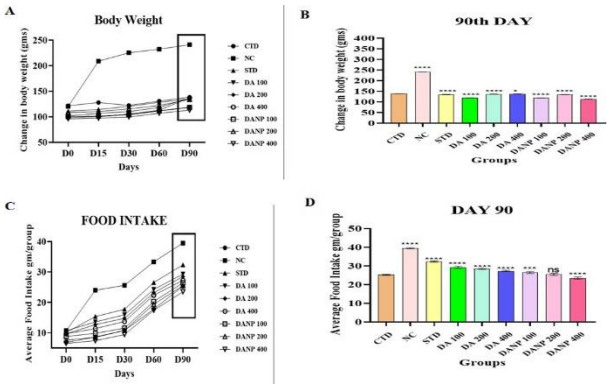

Fig no: 6: (A) % Gain in B.W in the investigational animals; (B) the change in B.W at 90th day when compared with Negative control (C) Intensity of Food Intake in the investigational animals;(D) Average Food Intake at 90th day when compared with Negative Control; n= 6 rats; * value of $p < 0.05$, radically diverged from the control group; * value of $p < 0.05$ radically diverged from group of negative control, treated groups. Values representing ± SD The statistical difference between the DA and DA loaded NP group treated group, control group, Negetive control group, Standard group (Atorvastatin), was analyzed by one way ANOVA followed by Dunnet test

Effect of DA concentrate and DA NP on profile of Lipid and level of Glucose, AI

The outcome of DA concentrates and DA NP on profile of lipid and level of glucose, AI showed in blood on experimental animals. The animals fed with HFD showed an increase in level total cholesterol, triglycerides, LDL, VLDL and atherogenic index (AI) where as there was a decline HDL level. The administration of different doses (100mg/kg, 200 mg/kg and 400 mg/kg body weight) of DA extract and DA NP in the experimental animals notably reduce these biochemical end point parameters next to normal level in dose dependent manner. The intensity of glucose in the HFD animals was reduced significantly by the administration of DA concentrate and DA NP in a dose of 100, 200 and 400 mg/kg Body weight when compared to negetive control group of animals. The data has been represented in fig: 7

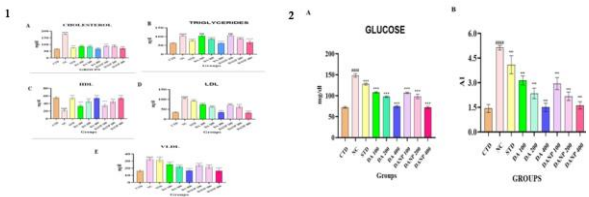

Fig no: 7 Effect of DA Extract and Nano-lipid Formulations on {1} Lipid profile (A. Cholesterol, B. Triglycerides, C. HDL, D. LDL, E. VLDL) {2} Glucose (A) and AI (B) in different experimental groups. Values are mean ± standard deviation; n=6 in each group; * p<0.05 ### value of p < 0.05, radically diverged from the control group; **** value of p < 0.05 radically diverged from group of negative control, treated groups. Values representing ± SD The statistical difference between the DA and DA loaded NP group treated group, control group, Negetive control group, Standard group (Atorvastatin), was analyzed by one way ANOVA followed by Dunnet test

Effect of Dolichos aciphyllus extract on antioxidant status

The effect of DA extract and the formulation in dose dependent manner has been represented in fig: 8. There was a significant increase in the levels of MDA in the hyperlipidemia induced animals with HFD in comparison to control group animals. When the animals treated with DA extract and the formulation, there was a significant reduction in the levels MDA. The level of GSH diminished in the HFD animals which was significantly went to normal levels in the treated animals. The antioxidant activity like SOD and Catalase (CAT) reduced in the disease control group where as the antioxidant activity in liver increased in the test animal group in dose dependent manner.

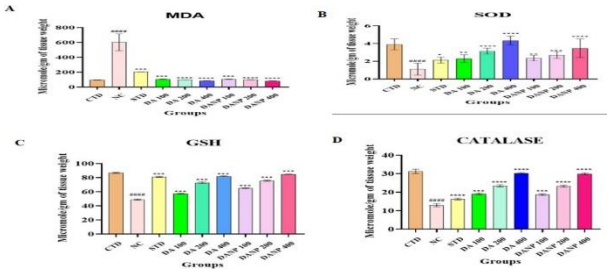

Fig no: 8 Effect of DA Extract and Nano-lipid Formulations on Antioxidant Profile like MDA (A); GSH (B); SOD (C) and Catalase (D) in different experimental groups Values are mean ± standard deviation; n=6 in each group; * p<0.05 ### value of p < 0.05, radically diverged from the control group; **** value of p < 0.05 radically diverged from group of negative control, treated groups. Values representing ± SD The statistical difference between the DA and DA loaded NP group treated group, control group, Negetive control group, Standard group (Atorvastatin), was analyzed by one way ANOVA followed by Dunnet test

3.7.4Effect of Dolichos aciphyllus concentrate on Liver Enzymes in blood & Creatinine, Urea and Liver Weight

The levels of liver enzymes were increased (p< 0.05) in the HFD group of animals when compared with Negetive control group. In the treated groups of animals the liver enzymes levels were decreased significantly using 100, 200 and 400 mg/kg body weight which was almost similar to the control group levels. The standard drug atorvastatin group in the dose of 10 mg/kg body weight showed significantly higher levels of AST and ALT when compared to control group of animals. According to the renal function test, there was significant change in the levels of creatinine and urea which became almost normal after treating with the DA extract and the DA extract prepared formulation. The treatment with DA Extract and DA NP and standard drug atorvastatin results in significant transformation of liver weight when compared with the Negative control group of animals. The acquired data was represented in the table: 6

Table no: 6 Effect of Dolichos aciphyllus concentrate & DA NP on Liver Enzymes in blood & Creatinine, Urea and Liver Weight

Groups	**AST(U/L)**	**ALT(U/L)**	**CREATININE**	**UREA**	**GGT(U/L)**	**LIVER WEIGHT(gm)**
Control	178.4 ± 0.7362	93.14 ± 0.98	0.6333 ± 0.01033	33.51 ± 0.824	8.92 ± 1.164	7.8383 ± 0.01169
Negative Control(HFD)	281.5± 0.82537####	217.96 ± .91####	2.7117 ± 1.02474####	103.2 ± 1.004####	56.81 ± 1.34####	15.795 ± 0.0468####
Standard(Atorvastatin)	190.3± 0.55571****	91.39 ± 1.22*	1.4817 ± 0.01472***	52.68 ± 0.855****	11.12 ± 1.014*	7.4667 ± 0.04082****
DA Extract (100mg/kg)	195.2± 1.23689****	118.1 ± .65****	1.1333 ± 0.02503****	56.91 ± 0.749****	36.26. ± 1.153****	8.84 ± 0.04243****
DA Extract (200mg/kg)	184.9± 0.94559****	101.15 ± .801****	0.9217 ± 0.01169****	54.83 ± 0.899****	29.33 ± 1.02****	8.9567 ± 0.01751****
DA Extract (400mg/kg)	182.5± 0.94498****	96.87 ± 1.475****	0.7717 ± 0.01169****	51.95 ± 0.832****	25.75 ± 1.37****	9.5333 ± 0.04676****
DA Extract NP	188.6 ±	115.483 ±	0.72 ±	36.91 ±	38.76 ±	7.8317 ±

(100mg/kg)	0.92104****	.905****	0.01265****	0.749****	.737****	0.01941
DA Extract NP (200mg/kg)	182.8 ± 0.84373****	97.182 ± .810****	0.8333 ± 0.01033****	34.83 ± 0.899	30.83 ± 1.431****	7.9533 ± 0.03724****
DA Extract NP (400mg/kg)	175.5 ± 1.16152****	93.955 ± .930	0.5717 ± 0.01169****	31.95 ± 0.832*	24.917 ± 1.157****	8.535 ± 0.02429****

The data are expressed as mean ±SD. The statistical difference between the DA and DA loaded NP group treated group, control group, Negetive control group, Standard group (Atorvastatin), was analyzed by one way ANOVA followed by Dunnet test

####Signifcant when compared to normal control ($p< 0.001$)
****Signifcant when compared to the Negetive Control group ($p< 0.001$)

Histopathological Studies:
The results from histological examination showed rigorous liver steatosis and swelling in the HFD treated experimental animal group, that were distinctly invalidated in the DA extract treated animal group and the formulation with high dose (400 mg/kg body weight). Larger size of adipose tissue has been observed in the HFD treated group while the size of the adipose tissue observed to be smaller in size in the groups when treated with DA extract and DA NP when compared to HFD treated group. The acquired results have been presented in fig: 10 & 11

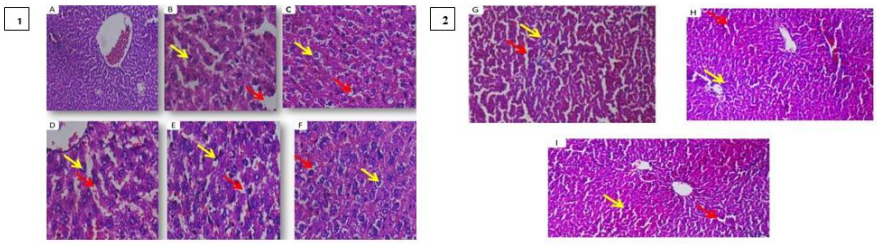

Fig No: 10 Hematoxylin and eosin (H&E) staining of liver tissues indicating the liver tissue structural design and droplets of liver. a: no damage liver in control group; b:fully damaged liver in HFD group of rats; c: recovered liver in Standard Drug (Atorvastatin) treated group of animals; d: mild healing of damaged liver in DA (100 mg/kg Body weight) treated group of animals e: moderate healing of damaged liver in DA (200 mg/kg Body weight) treated group of animals; f: momentous healing of damaged liver in DA (400 mg/kg Body weight) treated group of animals;

(2) g. mild healing of the damaged liver in DA Loaded Nanoparticles (100 mg/kg Body weight); h: moderate healing of damaged liver in DA Loaded Nanoparticles (200 mg/kg Body weight) with; i: momentous healing of damaged liver in DA Loaded Nanoparticles (400 mg/kg Body weight)

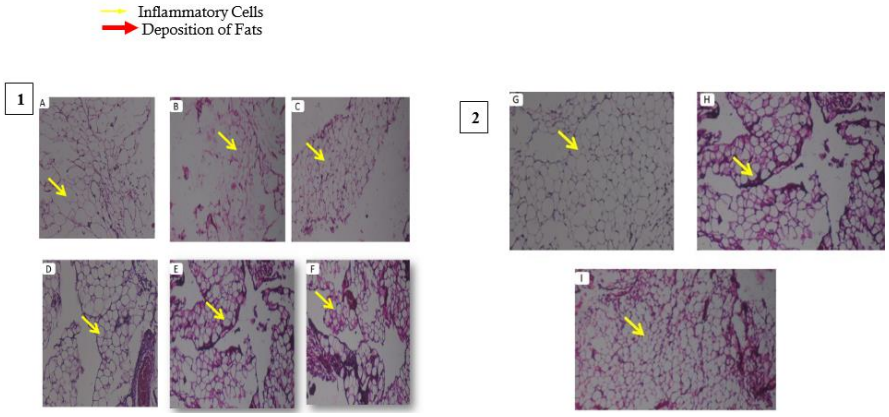

Fig no: 11 Histopathology of adipose tissue stained with Hematoxylin and eosin (H&E) stain of experimental rats; a: Normal Structural Design of adipose tissue in control group of rats; b: enhanced size of adipose tissue in negative control group of rats; c: reduced size of adipose tissue in group of animals treated with standard drug.

Atorvastatin d: mild decrease in size of adipose tissue in DA (100 mg/kg Body weight) treated group of animals; e:

moderate decrease in the size of adipose tissue in DA (200 mg/kg Body weight) treated group of animals; f: more decrease in size of adipose tissue in DA(400 mg/kg Body weight) treated group of animals.(2) g: mild decrease in the adipose tissue size with DA Loaded Nanoparticles(100 mg/kg Body weight) h. moderate decrease in the adipose tissue size DA Loaded Nanoparticles(200 mg/kg Body weight) i. the size of the adipose tissue has reached almost to normal like standard group(400 mg/kg Body weight).

Size of adiposities

DISCUSSION:

The disturbance in lipid metabolism is one of the primary factors for various diseases like CVS diseases, obesity, hypertension etc. The goal of the current investigation was to formulate safe & efficacious treatment to treat the lipid metabolism disturbances and to prevent several other diseases related to lipid metabolism disturbances in comparison to the marketed Hypolipidemic class of drugs. The phytochemical constituents present in the ethanolic extract of beans of Dolichos aciphyllus like Flavonoids, terpenoids, saponins, proteins, alkaloids etc was the key constituents which are accountable for the various therapeutic activity. Among all the phytochemical constituents present in the Dolichos aciphyllus beans, Flavonoids like Quercetin was notified through HPLC to be responsible for lowering the lipid profile as well as helps in bio transformation and excretion by removing cholesterol from the peripheral tissues to the liver (41). The DA extract could be useful for treating several metabolic disorders that are related to Hyperlipidemia. According to the results, DA extract contains a specified amount of heavy metals in accordance to the specifications which confirmed Dolichos aciphyllus plant was not been cultivated in a toxic environment. The results conclude that using DA extract for any treatment did not escort any heavy metal toxicity (42). The presence of any kind of microbial contamination could affect the quality and leads to toxic effect of the final formulation prepared with DA extract. So, the data indicates that the herbal DA extract did not show any presence of bacteria and the amount of yeast and moulds are in the specified limits (43).

DA extract has a remarkable in vitro antioxidant activity. This antioxidant activity in DA extract might be due to the content of Quercetin. Dolichos aciphyllus extract has a numerous Flavonoids like Quercetin, β- sitosterol, streptogenin, α- mannosides, β-glucosides (44). The presence of Flavonoid in the extract is the reason of highly efficient scavenging activity of the oxidizing molecules as well as several free radicals associated with various diseases (45).

The efficacy of ethanolic DA extract and the prepared formulation was notably more persuasive towards inhibition of pancreatic lipase and malic dehydrogenase enzyme which further reduce the synthesis and accumulation of lipids and fats in the body (46). The ethanolic DA extract and the DA formulation found to reduce the action of HMG CoA Reductase enzyme which plays a vital role in the synthesis of cholesterol which leads hyperlipidemia. Thus, the ethanolic extract of Dolichos aciphyllus was used for the Hyperlipidemic activity (47).

The DA extract and the prepared DA NP was noticed to be non-toxic and did not cause any mortality into the experimental animals' up to the dose of 4000 mg/kg after performing the acute oral toxicity. In accord with OECD rules 423 the dose in further in-vivo study was selected as1/5th and 1/10th dose used for the acute oral toxicity. So, the dose for the extract was finalized as 100mg/kg, 200 mg/kg and 400 mg/kg b.w for the further swot.

The b.w in HFD fed group of animals was significantly increased ($p< 0.05$) when analyzed against control group of experimental animals for a duration of 90 days. This increment of body weight was owing to building up of + (ve) energy in the High fat diet animals which lead to the increase in deposition of visceral fat. The group of animals medicated with DA concentrate showed a considerable decrease ($p< 0.05$) in b.w which was proportional to the dose in comparison to the High is fed diet fed animal group. The food intake by the animals is not the reason for the reduction in the b.w in the healed animal groups as the difference between the consumed food intake of the treated and HFD group of animals is not much significant. The reduction in body weight has might be due to its capacity to restrain lipogenesis and increased thermogenesis (48).

The negative control group which was induced with hyperlipidemia by HFD demonstrated a notable increase in plasma TC on evaluation with the reference group ($p< 0.05$). This previous research work proved the elevation in blood & tissue cholesterol and triglycerides with HFD (49, 50). The elevated concentration of cholesterol might be accumulated in the large blood vessels (51). The enhanced level of TG might be due to the reduction of lipoprotein lipase activity (52). DA Extract with the formulation (100mg/kg, 200 mg/kg and 400 mg/kg body weight) was proved to be remarkably decrease ($p< 0.05$) in cholesterol and triglycerides level in comparison along with the diseased group. The Flavonoid which is the primary Phytochemical constituent in the DA extract responsible for stimulating the activities of lipoprotein lipase leads to the diminution in cholesterol and triglycerides levels

& stimulates triglycerides uptake from the plasma through the skeletal muscle and adipose tissue (53). The standard group treated with atorvastatin (10mg/kg) also explained momentous decline ($p< 0.05$) in cholesterol and triglycerides levels in plasma and the tissue.HFD fed animals group showed an elevation ($p< 0.05$) in LDL and VLDL levels where as there was no notable augment in the levels of HDL. The model group which is treated with Atorvastatin (10mg/kg) illustrated that there is significant decrease ($p < 0.05$) in LDL and VLDL levels. On the other hand, it did not show any enhancement in HDL levels. Group treated with the DA extract and DA NP (100mg/kg, 200 mg/kg and 400 mg/kg b.w) radically lessen ($p< 0.05$) LDL and VLDL levels. The decrease level in the LDL leads to reduction in the coronary heart disease rate (54). The DA extract and DA NP (100mg/kg, 200 mg/kg and 400 mg/kg body weight) healed group of animals demonstrated an elevation in HDL levels. There can reduce rate of morbidness and loss of life in CVS patients with the increase in the HDL concentration (55).

The serum ALT and AST level was reduced significantly with remedy of DA extract and DA NP when compared with HFD negative control group. The achieved discovery was similar to previous study results (56).

The levels of creatinine and urea were significantly reduced when treated with DA Extract and the formulations in comparison to the negative control group of animals. The outcomes matched with the previously researched data (57.58).

The accumulation of foam cells or fatty infiltration or lipids in heart, coronaries, liver and kidney has been due to Atherogenic index (AI). The threat to these organs will increase as there will be increase in AI. The danger of cardiovascular disease can be predicted by measuring AI (TG/HDL) (59). DA extract has been significantly decreases the AI leading to various advantageous results of the Flavonoids present in the DA extract in treating cardiovascular diseases.

The free radicals which are responsible for hastening then oxidative squalor of polyunsaturated fatty acids inside the membrane as well as there was enhancement in the level of lipid peroxides and hydro-peroxides

(60). MDA (Malanoaldehyde) is the important biomarker for analyzing oxidative damage as it is the primary product of lipid peroxidation (61).It was proved that amplified MDA levels leads to enhanced lipid peroxidation in the hyperlipidemia induced rats but raised MDA level is not the reason of hyperlipidemia

(62). The generation of free radicals and endogenous antioxidant defense mechanism balance results in the peroxidation degree by free radicals. A reduction in level of GSH, enhancement in lipid per-oxidation and endogenous antioxidant enzymes such as CAT (Catalase) and SOD (Superoxide Dismutase) activities impairment was related to oxidative stress (63). The experimental group treated with Atorvastatin confirmed a fall in the production of free radicals as well as increase the level of GSH and SOD but reduce the level of MDA in tissue (65). DA extract & DA NP treated group repealed a variation in the markers of oxidative stress & raised the level of GSH, diminishing MDA and stimulate SOD and CAT activities in the HFD induced Hyperlipidemia experimental rats. The acquired data proved that the DA extract and DA NP were capable of increasing the antioxidant defense mechanism leads to the protection of liver from the oxidative stress as SOD and CAT are the primary enzymes in the enzymatic antioxidant defense system. The superoxide anion was eliminated by SOD which was transferred into hydrogen peroxides followed by reduction in toxic level where as superoxide anion was broken down to hydrogen peroxide by Catalase. So, the acquired data revealed that DA extract and its formualation has efficient anti-oxidant activity and has good potential and beneficial against hyperlipidemia.

There was significant change in the weight of liver in the hyperlipidemia induced negative control group. The treatment groups showed significant reduction in the liver weight in the dose of 100,200 and 400 mg/kg body weight when compared with the negetive control group.

The results of histopathological study illustrated that the liver cells of the hyperlipidemia induced group has been damaged which was recovered and improved in the DA extract and its formulation treated group. Lipid metabolism disorders may be due to abnormal production of fatty acid and deterioration (65).The treated groups have also achieved reduced degree of vascularization in comparison to the hyperlipidemia induced group. The centrilobulor region of liver has several foci of inflammations as well as inflammatory region has infiltration of inflammatory cells. Inside sinusoidal space of the liver there was less to higher sinusoidal space dilation and in the centrilobular region inflammation followed by infiltration of inflammatory cells was observed in the HFD fed group of experimental animals.

Limitation of the Research Study:

The limitations of the current research work were faced with the safety, quality and lack of reporting of the herbal drug. The solubility of the herbal drug was also been a major limitation faced in the present piece of work. The use of single animal model in the study may fail to reproduce pathophysiology of human liver. The above mentioned limitations can be

rectified by formulating the herbal drug into a targeted drug delivery system in future. Furthermore, representation of clinical data is necessary so that the safety and efficacy of the DA Extract and DA NP can be easily explained.

CONCLUSION

The contemporary research work analyzed the anti-hyperlipidemic activity of Dolichos aciphyllus (DA) extract and Dolichos aciphyllus loaded formulation (DA NP) on the high fat diet induced hyperlipidemia in female wistar albino rats. The DA Extract found to be efficacious in the inhibition of the activity of HMG CoA Reductase enzyme which is the key element in the increase of cholesterol level and also able to inhibit pancreatic lipase and malic dehrogenase activity which helps in reducing the cholesterol level in the blood serum thus making the DA extract more potent for treating Hyperlipidemia. The current research work can be concluded that high dose of DA extract and DA NP has a good capacity to lower the enhanced lipid profile which was similar to the standard drug (Atorvastatin). Additionally, from the research work it was also proved that DA extract and DA NP has an excellent anti-oxidant activity which can efficiently decrease the risk of CVS disorder. Additionally, histopathological examination proved changes in disordered hepatic tissue, changes in fatty liver and the changes in the dimension of the adipose tissue in the High fat diet experimental animals when compared with the standard group of animals. Thus, the beans of DA extract and its formulation was proved to be demonstrated potential anti-hyperlipidemic agent in addition to the existing conventional Hyperlipidemia treatment.

Ethics Approval and participation assent:

The current research work has been performed under the necessary ethical guidelines of the board for the reason of caring and management of the experimental animals after receiving the approval from the organizing members with the CPCSEA approval no (CPCSEA Number ITS/02//IAEC/2022).

Funding Declaration:

There was no funding available for the current research activity Acknowledgement:

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Authors' Contribution:

Each and every investigator has significantly added in the present investigation to complete the manuscript. Moumita Barman has performed all the pharmacological activities, literature survey and writing the manuscript. Dr. Praveen Gaur helped in the interpretation of the results. Dr. Dheeraj Nagpal evaluated the manuscript. All the authors reviewed and gave their consent for concluding the present piece of work. The authors confirm that no paper mill and artificial intelligence was used.

Conflict of Interest:

The researchers have no divergence of interest financially or in other matters.

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