Journal of Biomedical and Pharmaceutical Research
Available Online at www.jbpr.in
CODEN: - JBPRAU (Source: - American Chemical Society)
PubMed (National Library of Medicine): ID: (101671502)
Index Copernicus Value 2018: 88.52
Volume 8, Issue 6: November-December: 2019, 41-50
ISSN (Online): 2279-0594
ISSN (Print): 2589-8752

Research Article

ANTIHYPERLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF *AZADIRACHTA INDICA*

*** Shalu Baghel[1], Charanjeet Singh[2]**
[1]Department of Pharmacology, Jaipur College of Pharmacy, Jaipur, Rajasthan, India
[2]Principal, Biyani Institute of Pharmaceutical Sciences, Champapura, Jaipur, Rajasthan, India

Article Info: Received 10 September 2019; Accepted 14 November. 2019
DOI: https://doi.org/10.32553/jbpr.v8i6.678
Address for Correspondence: Shalu Baghel
Conflict of interest statement: No conflict of interest

ABSTRACT:
Hyperlipidemia often results from delayed or defective clearance, or overproduction of VLDL by the liver, which is subsequently transformed into LDL. The whole plant of *Azadirachta indica* was collected from local areas of Jaipur district areas of Rajasthan. The plant is authenticated by university of rajasthan by Drmanju Sharma dept of botany Selected medicinal plant were cut into small pieces, cleaned and shade dried at room temperature then subjected to physical evaluation with different parameters. Macerated 5 g of air dried drug coarsely powdered with 100 ml of ethanol of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 h. About 2-3 g of powder is accurately weighed in a china dish and kept in hot air oven maintained at 110±1°C for four hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained. For insoluble ash value Boiled the ash for 5-10 minutes with 25 ml of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible washed with hot water, ignited and weighed. Whole plant of ***Azadirachtaindica***was reduced to fine powder and was subjected to maceration in a 1 liter conical flask for 7 days at room temperature with alcohol (70%) water (30%). In the present study we aimed to screen various extracts of selected plants on Cholesterol induced hyperlipidemic rat model. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyerlipidemic activity in atherogenic diet induced hyperlipidemic rats and led to the development of new Herbal formulation possessing antihyperlipidemic and antiatherosclerotic activities. This is the first study which investigates the hypolipidemic activity.
Keywords: *Azadirachta indica,* antihyperlipidemic, antiatherosclerotic activities, Maceration.

1. INTRODUCTION:

Hyperlipidemia is a secondary metabolic dysregulation associated with diabetes. Besides the cause effect relationship with diabetes, elevated serum level of triglycerides, cholesterol and LDL are major risk factors for the premature development of cardiovascular disease like arthrosclerosis, hypertension, coronary heart disease etc[1] Hyperlipidemia often results from delayed or defective clearance, or overproduction of VLDL by the liver, which is subsequently transformed into LDL. Hypercholesterolemia involves defective hepatic and nonhepatic LDL receptors. Excess intake of saturated fats increases the liver's production of VLDL and triglycerides via a molecular mechanism involving protein activators. Saturated fats are found in animal products, such as meat, whole milk dairy products (milk, cream, cheese), and butter, and tropical oils (palm, palm kernel, and coconut).

1.1 Types of hyperlipidemia

Depending on the complexity of the disease, Hyperlipidemia classified into two types.

a . Primary Hyperlipidemia.
b. Secondary / Acquired Hyperlipidemia.

a. Primary Hyperlipidemia:

Several genetic conditions are known to responsible for primary Hyperlipidemia, such as lipoprotein lipase deficiency, apolipoprotein C-II deficiency etc. The primary hyperlipidemia may be treated by anti-lipidemic drugs. Primary Hyperlipidemia are again classified into 5 types.

- Type-I Hyperlipidemia: Severe elevation of chylomicrons (CMs) with resultant elevation of TGs.

- Type-II (A) Hyperlipidemia: Elevations of LDL – C only.
- Type-II (B) Hyperlipidemia: Elevations of both LDL-C and triglycerides (TG's).
- Type-III Hyperlipidemia: It develops due to defect in VLDL remnant Clearance.
- Type-IV Hyperlipidemia: It is characterized by hyper TG's
- Type-V Hyperlipidemia: Characterized by elevated levels of CMs and VLDL.

b. Secondary Hyperlipidemia

In this many factors can influence the level of TGs in circulation like diabetes, obesity etc. Secondary Hyperlipidemia demands treatment of original diseases rather than Hyperlipidemia.

1.2 Causes of secondary Hyperlipidemia:

- Metabolic influences: Diabetes, obesity, hyperuricemia, glycogen storagediseases.
- Harmonal influences: Insulin, estrogen, thyroxine
- Nutritional influences:-Alcohol, high carbohydrate intake
- Disease states:-Renal diseases, renal failure, nephrotic syndrome
- Drugs: - Diuretics

2. MATERIAL AND METHODS:

2.1 Selection of plants

The whole plant of ***Azadirachtaindica***were collected from local areas of Jaipur district areas of Rajasthan. The plant is authenticated by university of rajasthan by Drmanju Sharma dept of botany Selected medicinal plant were cut into small pieces, cleaned and shade dried at room temperature then subjected to physical evaluation with different parameters. Then these selected medicinal plant was subjected to size reduction to get coarse powder, separately, in a mechanical grinder and then passed through sieve no. 40 to get desired particle size and stored in well closed glass jars. Then uniform powder was subjected to standardization with different parameters.

2.2 Procedure for different parameters

2.2.1 Physical test:

Nature : Course Powder
Coloue : Dark Brown
Odour : No odour
Taste : Bitter

2.2.2 Determination of alcohol soluble extractive

Macerated 5 g of air dried drug coarsely powdered with 100 ml of ethanol of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 h. filtered rapidly taking precautions against loss of ethanol. Evaporated to dryness in a tared flat bottom shallow dish, dried at 105±1°C and weighed.Calculated the percentage of alcohol soluble extractive with reference to the air dried drug.

2.2.3 Determination of water soluble extractive

Macerated 5 g of air dried drug coarsely powdered with 100 ml of water in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 h. filtered rapidly taking precautions against loss of chloroform water. Evaporated to dryness in a tared flat bottom shallow dish, dried at 105±1°C and weighed.Calculated the percentage of water soluble extractive with reference to the air dried drug.

2.2.4 Determination of benzene soluble extractive

Macerated 5 g of air dried drug coarsely powdered with 100 ml of benzene of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 h. filtered rapidly taking precautions against loss of chloroform water. Evaporated to dryness in a tared flat bottom shallow dish, dried at 105±1°C and weighed.Calculated the percentage of water soluble extractive with reference to the air dried drug.

2.2.5 Determination of Petroleum ether extractive

Macerated 5 g of air dried drug coarsely powdered with 100 ml of petroleum ether of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 hrs. filtered rapidly taking precautions against loss of chloroform water. Evaporated to dryness in a tared flat bottom shallow dish, dried at 105±1°C and weighed.Calculated the percentage of water soluble extractive with reference to the air dried drug.

2.2.6 Determination of Hydro-alcohol extractive

Macerated 5 g of air dried drug coarsely powdered with mixture of 70 ml of ethanol and 30 ml water in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for 18 h. filtered rapidly taking precautions against loss of ethanol. Evaporated to dryness in a tared

flat bottom shallow dish, dried at 105±1°C and weighed.Calculated the percentage of alcohol soluble extractive with reference to the air dried drug.

2.3 Loss on drying

About 2-3 g of powder is accurately weighed in a china dish and kept in hot air oven maintained at 110±1°C for four hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

$$\text{Loss on drying } (\%) = \frac{\text{Loss of weight}}{\text{Weight of the drug in gms}}$$

2.4 Determination of ash value

About 2-3 g weighed crude drug powder in a tarred silica dish was ignited and weighed. Scattered the powder drug on bottom of the dish, incinerated by gradually increasing the heat not exceeding dull red heat until free from carbon, cool

$$\text{Total ash value of sample } \% = \frac{z - x}{y} \times 100$$

and weighed.

Where,
x = weight of empty dish, y =weight of drug taken, z =weight of the dish + Ash (after complete incineration)

2.5 Total ash value

2.5.1 Determination of acid insoluble ash

Boiled the ash for 5-10 minutes with 25 ml of diluted hydrochloric acid, collected the insoluble matter in a Gooch crucible washed with hot water, ignited and weighed. Calculated the percentage of acid insoluble ash with reference to air dried drug.

$$\text{Acid insoluble ash value of the sample } \% = \frac{a}{y} \times 1($$

Where, a = weight of the residue, y= weight of air dried drug

2.5.2 Determination of water soluble ash

Boil the ash for 5-10 minutes with 25 ml of water, collected the insoluble matter in a Gooch crucible, washed with hot water and ignited to constant weight at a low temperature. Subtracted the weight of insoluble matter from the weight of ash. The difference in weight represents the water soluble ash. Calculated the percentage of water soluble ash with reference to the air dried drug. Water soluble ash – Weight of insoluble matter

$$\text{Percentage of water soluble ash} = \frac{a}{b} \times 100$$

Where, a = water soluble ash, b = air dried drug

2.6 Preparation of alcoholic extract of *azadirachtaindica*

In the present study whole plant of ***Azadirachtaindica***was reduced to fine powder and was subjected to maceration in a 1 liter conical flask for 7 days at room temperature with alcohol (70%) water (30%). The flasks were securely plugged with adsorbent cotton and was shaken periodically till complete maceration. After the complete extraction, the solvent was distilled off and concentrated on water bath to a dry residue. This residue used as drug for present study.

2.7 Preliminary phytochemical investigation of extracts

Qualitative chemical tests of hydro-alcoholic extracts of *Azadirachtaindica*was conducted to identify the various phytoconstituents. The various tests and reagents used are given below and observations are recorded.

2.8 Pharmacological evaluation

- Wistar albino rats of either sex (150-200gm)
- Methanolic extract of *A.INDICA*
- High Cholesterol diet pellets
- 5% Aqueous gum acacia

Animal models:Wistar albino rat

Standard drug: Atorvastatin- drugs

3. EXPERIMENTAL PROCEDURE:

In the present study we aimed to screen various extracts of selected plants on Cholesterol induced hyperlipidemic rat model[33,34]

Healthy Wistar albino rats weighing between 150-200gm were acclimatized to the laboratory at temperature (25±1)^{0}c, relative humidity(50±15) %, 12hrs light-dark cycles, kept in standard polypropylene cages and given standard diet and water *ad-libitum*. The animals were divided into control, toxic, standard and test groups of A. Indica 100mg/kg, A. Indica200mg/kg, b.wp.o, suspended in 5% gum acacia solution, daily once .Each comprising of 6 animals in all sets of experiments. Animals in the normal control group, received normal saline orally. Except control group rest

other groups were fed with rich cholesterol diet pellets supplied by M/s Rayans biotechnologies Pvt.Ltd., Hyderabad. Standard group received Atorvastatin 10mg/kg b.wp.o suspended in 5% gum acacia solution. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. Feeding the animals with cholesterol supplied diet induces hyperlipidemia, especially hypercholesterolemia and hypertriglyceridemia.

Table :1 Protocol for study of antihyperlipidemic activity using Leavesmethanolic extracts of *AzadirachtaIndica* in albino rats[35]

Group	Treatment (20)days
Group I	Normal saline
Group II	Cholesterol diet
Group III	Cholesterol diet + Atorvastatin (10mg/kg b.w) suspended in
	5% gum acacia solution
Group IV	Cholesterol diet + A. Indica (100 mg/kg b.w) suspended in
	5% gum acacia solution
Group V	Cholesterol diet + A .Indica (200 mg/kg b.w) suspended in
	5% gum acacia solution

The animals were divided into control, toxic, standard and test extracts of A. Indica 100mg/kg, A. Indica 200mg/kg,.Each comprising of 6 animals in all sets of experiments. Animals in the normal control group received normal saline orally. Except control group rest other groups were fed with rich cholesterol diet pellets .Standard group received atorvastatin 10mg/kg orally. The treatment was given for 20 days. In between mean body weight of the animals was checked time to time. On 21st day the blood samples were withdrawn from the arterial damage. Total cholesterol (TC), triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) were analysed from serum.

3.1 Biochemical estimations[36]

At the end of experimental period, rats were anesthetized with ether. Blood samples were collected by cardiac puncture method. Serum total cholesterol, triglycerides, high density lipoproteins-cholesterol using beacon diagnostic Pvt ltd kits. Serum LDL, VLDL was determined by calculation.

3.2 Procedures for testing parameters[36]

3.2.1 Estimation of serum of triglycerides

Diagnostic kit was used for estimation of triglycerides, which followed end point colorimetry enzymatic test using glycerol-3-phosphate oxidase.

Table 2: Reagents used

S. No.	Reagent composition	Conc. in the final test mixed
1.	Pipes butter	50mmol/l
2.	4-Chlorophenol	5mmol/l
3.	Mg 2+	5mmol/l
4.	ATP	1 mmol/l
5.	Lipase	> 5000 U/l
6.	Peroxidase	>1000 U/l
7.	Glycerol Kinase	>400 U/l
8.	Glycerol - 3-phosphate oxidase	>4000 U/l

Standard: The concentration of standard triglyceride used was 200mg/dl

Table 3: Reaction Parameters

1.	Reaction type	End point
2.	Wave Length	505nm
3.	Optical Length	1 cm
4.	Temperature	37*C
5.	Measurment	Against reagent blank

Table 4: Summary of assay Details

Pipetted in to test tube	Blank	Standard	Test
Reagent	1000 µl	1000 µl	1000 µl
Standard Triglyceride (200mg/dl)	-	10 µl	-
Sample (serum)	-	-	10 µl

Calculations: Serum triglycerides (mg/dl) = Abs of test **/** Abs of STD × Conc of standard**.**

3.2.2 Estimation of serum total cholesterol[37]

The reagents kits intended for the *In-vitro* quantitative determination of cholesterol in serum/plasma

Table 5: Process for estimation of total cholesterol levels

	Procedure for 1ml			Procedure for 3ml		
	B	S	T	B	S	T
Enzyme Reagent	1 ml	1 ml	1 ml	3 ml	3 ml	3 ml
Standard Cholesterol (200mg/dl)	-	10µl	-	-	30µl	-
Sample(serum)	-	-	10µl	-	-	30µl

Procedure: Bring all the reagents of assay to room temperature. Mix well and incubate for 5 min at room temperature. Mix well and measure the absorbance of standard and test against the reagent blank at 505 nm.

3.2.3 Estimation of serum high-density lipoprotein cholesterol (hdl-c)[38]

Diagnostic kit was used for estimation of HDL cholesterol, which followed Cholesterol oxidase / peroxidase (CHOD-POD) method

Table 6: Reaction parameters

1.	Reaction type	End point
2.	Wave Length	505nm
3.	Optical Length	1 cm
4.	Temperature	37*C
5.	Measurment	Against reagent blank

Preparation: Take 0.5 ml of serum /plasma in to glass tube. Add 50 µl precipitating reagent. Mix well, leave it at R.T. For 10 min. centrifuge at 3000 r.p.m. for 10 min, take the clear supernatant for HDL cholesterol estimation

Table 7: procedure for cholesterol estimation

	B	S	T
Enzyme reagent	1 ml	1 ml	1 ml
Standard	-	0.01 ml	-
Supernatant sample	-	-	0.01 ml

Mix well and incubate for 5 min at 37°c. Measure the absorbance of HDL& STD at 510 nm.

Table 8: Effect of *a. Indica*methanolic extracts on body weight on hyperlipidemic rat models

Days	Mean body weight(gm) change in body weight				
	Normal	Cholesterol	STD	A. Indica (100mg/kg)	A. Indica (200mg/kg)

0th day	140	142	143	142	140
5th day	143	149	145	152	144
10th day	148	167	152	158	150
15th day	151	175	160	174	156
20th day	155	199	170	196	168

The body weight of rats increased from the 0th day to 20th day. There is a significant increase in the body weight of cholesterol treated rats, when compare to the normal rats. *A Indica*methanolic extract of 100mg/kg b.wp.o treated group also showed significant decrease in the body weight, when compare to the normal rats. Less significant increase in the body weight of standard,.

4. RESULT AND DISCUSSION:

4.1 Physico-chemical characterization of *azadirachta indica*

S.No.	Parameters	Observations
I	Physical Tests	
	Nature	Coarse Powder
	Color	Dark Brown
	Odor	No odor
	Taste	Bitter
II	Extractive Values	
	Aqueous	14.38
	Alcohol	16.80
	Hydro – Alcoholic (70:30)	20.31
	Benzene	13.45
	Petroleum Ether	16.72
III	Loss on Drying	4.8
IV	Ash Values	
	Total Ash	11
	Acid Insoluble Ash	1.5
	Water Soluble Ash	0.5

The % yield of extraction value for physico-chemical characterization of AzadirachataIndica was calculated:

- Aqueous 14.38%
- Alcohol 16.80%
- Hydro-Alcoholic 20.31%
- Benzene 13.45%
- Petroleum Ether 16.72%

The % yield of LOD for physico-chemical characterization of AzadirachataIndica was calculated:

- LOD 4.8%

The % yield of ash value for physico-chemical characterization of AzadirachataIndica was calculated:

- Total ash 11%
- Acid insoluble ash 1.5%
- Water soluble ash 0.5%

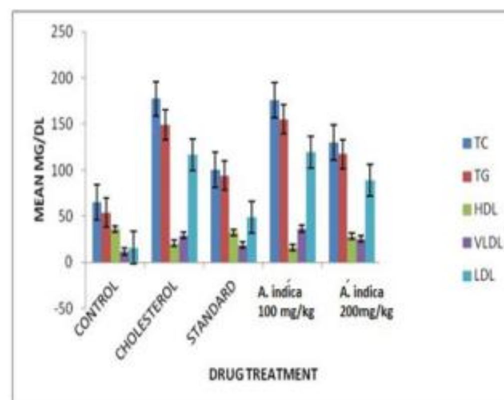
4.2 Preliminary phytochemical investigation of ethanolic extracts of*azadirachtaindica*

S.No.	Name of the test	Observation
I	Test for Carbohydrates	-
	Molisch's test	-
	Fehling's test	-
	Benedict's test	-
	Barfoed's test	-
	Cobalt-Chloride test	-
	Test for non-reducing sugar	
II	Test for Proteins	+
	Biuret test	+
	Million's test	+
	Xanthoproteintets	+
	Test for protein containing sulphur	+
	Precipitation test	
III	Test for Steroids	
	Salkowski reaction	+
	Liberman-Burchard	+
	Liberman's reaction	+

IV	Test for Amino acids	+
	Ninhydrin test	+
	Test for tyrosine	+
	Test for tryptophan	
V	Test for Flavonoids	+
	Shinoda test	+
	Lead acetate test	+
	Alkaline solution test	+
	Ferric chloride test	
VI	Test for Alkaloids	
	Dragendroff test	+
	Mayer's test	+
	Hager's test	+
	Wagner test	+
VII	Test for Tannins	
	Lead acetate test	+
	5 $FeCl_3$ test	+
	Gelatin solution	+
	Bromine water	+
VIII	Test for Vitamins	
	Test for Vitamin A	-
	Test for Vitamin C	+
	Test for Vitamin D	-
IX	Test for Glycosides	-
	Beljet's test	-
	Legal's test	-
	Keller killani test	-
	Libermann's test	-
	Foam test	
	Fluorescence test	
X	Test for Triterpenoids	-
	Salkowaski test	-
	Liebermann Burchardt test	

The phytochemical investigation of ethanolic extracts was possitive in protioens, steroids, amino acids, flavanoids, alkaloids, tannins and vit c.

4.3 Graphical representation of *a. Indica* extracts on cholesterol diet induced Hyperlipidemic model in wistar albino rat



4.4. Cholesterol induced hyperlipidemia: Effect of administration of selected Leaves extracts (100 mg/kg, p.o., once daily) /Atorvastatin (10mg/kg, p.o, once daily) on serum lipid Parameter levels in rats fed with Cholesterol Diet for 20days.

4.5 Effect on serum total cholesterol (serum tc) level

- Rats fed with Cholesterol for 20 days had serum TC level of (177.20±2.698 mg/dl) when measured on day 21. This was significantly higher ($p<0.001$) when compared to serum TC levels in normal control rats (64.89±2.280 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o., once daily) had serum level of 100.22±0.9657 mg/dl when measured on day 21. This was significantly lower ($p<0.001$) when compared to the serum TC levels in Cholesterol treated toxic control groups (177.20±2.698 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with A. Indica 100mg/kg, A. Indica 200mg/kg, had serum TC level of 175.61±2.314, 130.23±1.641 mg/dl respectively when measured on day 21. These values were significantly lower ($p<0.001$) when compared to the serum TC level in Cholesterol control rats (177.20±2.698 mg/dl).

4.6 Effect on serum triglyceride (serum tg) level

- Rats fed with Cholesterol for 20 days had serum TG level of (149.13±2.165 mg/dl) when measured on day 21. This was significantly higher ($p<0.001$) when compared to serum TG levels in normal control rats (53.90±1.66 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o., once daily) had serum level of (93.95 ± 1.205 mg/dl) when measured on day 21. This was significantly lower ($p<0.001$) when compared to the serum TG

levels in Cholesterol rats(toxic group) (149.13±2.165 mg/dl).

- Cholesterol induced hyperlipidemic rats treated with A. Indica 100mg/kg, A. Indica 200mg/kg, b.wp.o, once daily, had serum TG level of 155.12 ±1.321, 117.24±2.464 and 140.21±2.314 mg/dl respectively when measured on day 21. These values were significantly lower ($p<0.05$) and ($p<0.001$) when compared to the serum TG level in Cholesterol group (149.13±2.165 mg/dl).

4.7 Effect on serum hdl cholesterol (serum hdl-c) level

- Rats fed with Cholesterol for 20 days had serum HDL-C level of (20.71±1.221 mg/dl) when measured on day 21.This was significantly lower ($p<0.001$) when compared to serum HDL-C levels in normal control rats (36.15±1.125 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with atorvastatin (10mg/kg, p.o. once daily) had serum HDL-C level of 32.51±0.7098 mg/dl when measured on day 21.This was significantly higher ($p<0.001$) when compared to the serum HDL-C levels in Cholesterol control rats (20.71±1.221 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with A. Indica 100mg/kg, A. Indica 200mg/kg, b.wp.o, once daily, had serum HDL-C level of 16.23 ± 0.148, 28.11 ± 0.631 and 20.14 ±0.145 mg/dl respectively, when measured on day 21.These values were significantly higher ($p<0.05$) and ($p<0.001$) when compared to the serum HDL-C level in Cholesterol control rats(20.71±1.221 mg/dl).

4.8 Effect on serum vldl cholesterol (serum vldl-c) level

- Rats fed with Cholesterol for 20 days had serum VLDL-C level of (29.23±0.4326 mg/dl) when measured on day 21.This was significantly higher ($p<0.001$) when compared to serum VLDL-C levels in normal control rats (11.76±0.3387 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with Atorvastatin (10mg/kg, p.o) had serum VLDL-C level of 18.78±0.2407 mg/dl when measured on day 21, showing lower significant change ($p<0.001$) when compared to the serum VLDL-C levels in Cholesterol control rats (29.23±0.4326mg/dl).
- Cholesterol induced hyperlipidemic rats treated with A. Indica 100mg/kg, A. Indica 200mg/kg, b.wp.o, once daily, had serum VLDL-C level of 36.41±0.552, 25.34±0.414 and 30.24±0.326 mg/dl respectively, when measured on day 21. These values were significantly lower ($p<0.05$) and ($p<0.001$) when compared to the serum VLDL-C level in Cholesterol control rats (29.23±0.4326 mg/dl).

4.9 Effect on serum ldl cholesterol (serum ldl-c) level

- Rats fed with Cholesterol for 20 days had serum LDL-C level of (116.26±3.507 mg/dl) when measured on day 21.This was significantly higher ($p<0.001$) when compared to serum LDL-C levels in normal control rats (16.00±2.656 mg/dl).
- Cholesterol induced hyperlipidemic rats treated with Atrovastatin (10mg/kg, p.o., once daily) had serum LDL-C level of 48.89±0.7986 mg/dl when measured on day 21.This was significantly lower ($p<0.001$) when compared to the serum LDL-C levels in Cholesterol control rats 116.26±3.507 mg/dl).
- Indica 100mg/kg, A. Indica 200mg/kg,b.wp.o, once daily, had serum LDL-C level of 119.63±0.143, 88.61±0.241 and 110.23±0.341 mg/dl respectively, when measured on day21.These vales were significantly lower ($p<0.001$) when compared to the serum LDL-C level in Cholesterol control rats (116.26±3.507 mg/dl).

5. CONCLUSION

From this work we conclude that A.Indica plants posses ability to decrease cholesterol levels in the body. A.Indica ethanolic extract possess highly significant action towards reducing the body cholesterol. Hence the folklore usage has been validated. A.Indica can be treated as Nutraceuticals. Preliminary phytochemical investigations showed the presence of bioactive compounds like glycosides, sterols, terpenoids and phenolic compounds in Azadirachta indica. The plant extracts showed no toxicity at a maximum dose of 3000 mg/kg. All the extracts tested showed significant antioxidant activities in DPPH, Nitric oxide and lipid peroxidation methods in a dose dependant manner. All the extracts tested *in-vivo* showed decrease in lipid profiles and atherogenic index in a dose dependent manner.. The decrease in the levels of hepatic enzymes ALT, AST, ALP and lipid peroxidation indicate the hepatoprotective

nature of the extracts. The alcoholic extracts of selected herbal drugs could be formulated into effective hypolipidemic dosage form. Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyerlipidemic activity in atherogenic diet induced hyperlipidemic rats and led to the development of new Herbal formulation possessing antihyperlipidemic and antiatherosclerotic activities. This is the first study which investigates the hypolipidemic activity.

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