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Research Article

EVALUATION OF ANTI HYPERLIPIDEMIC ACTIVITY OF ETHANOLIC EXTRACT OF AMARANTHUS ROXBHURGIANUS IN ALBINNO RATS

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Abstract:

Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG) concentrations, and a decrease in the high-density lipoprotein (HDL) concentration. The aim of the present study is to promote a potential new herbal formulation to prevent atherosclerosis at a low cost. In this study ethanolic extract of Amaranthus roxbhurgianus- showed positive to following phytochemical constituent's Carbohydrates, Glycosides, Flavonoids, Proteins, Sterols. The significant reduction in serum cholesterol,TG,LDL, ,AI, and also increased in total HDL level in the different dose level(100,200 mg/kg) in high cholesterol diet. Hence it is going to be concluded that the potential benefits of the extracts of Amaranthus roxbhurgianus has been demonstrated well in advance and can be used further to demonstrate the antihyperlipidemic as well as controlling of both triglyceride levels and reducing the risk of factors of cholesterol inducers. The aforementioned results of the research suggest that the Amaranthus roxbhurgianus found to have the potential antihyperlipidemic action. **Keywords:** Anti-hyperlipidemic activity, Amaranthus Roxbhurgianus, Albino rats

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INTRODUCTION: HYPERLIPIDEMIA:

Hyperlipidemia is a disorder of lipid metabolism manifested by increase of plasma concentrations of the various lipid and lipoprotein fractions such as increase of serum total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG)concentrations, and a decrease in the high- density lipoprotein (HDL) concentration. Hyperlipidemia is the key risk factor for cardiovascular disorders and has been reported as the most common cause of death in developed as well as developing nations.[1-2] Hyperlipidemia may be caused by specific genetic abnormalities called primary hyperlipidemia or may be idiopathic caused by lifestyle habits or medical diseases such as diabetes, kidney disease, pregnancy, hypothyroidism and heart disease. [3—6]

Amaranthus roxburghianus has spread through tropical and subtropical latitudes around the world. It is an annual plant growing to 0.6 m that is widely distributed in the humid zone. The plant grows wild in cultivated fields, waste places, roadsides, garbage heaps and abandoned fields. It will grow both in wet or dry sites, but grows best when soil moisture levels are below field capacity. [7] It is being commercially cultivated in West Bengal, Maharashtra and Tamil Nadu, Andra Pradesh, Telangana.

The developments of the newly born chemical and pharmaceutical industry have brought about great social enthusiasm. The on-going discovery of more and more powerful new medicines, though not less toxic, seems to promise a bright future in which there is a specific pharmacological product to treat every disease. The rational and scientific use of plants. based on chemical and pharmacological research, is truly the only way to correctly use medicinal herbs. Furthermore, the efficiency of medicinal herbs increases when they are used within the frame of natural revitalizing treatment. The aim of the present study is to promote a potential new herbal formulation to prevent atherosclerosis at a low cost. The objectives are to conduct a literature survey for establishing the relevance of the study.⁸⁻¹³ The objectives are to estimate the active component from Ethanolic extract of Amaranthus roxbhurgianus, Evaluating the acute toxicity studies by using mice and rats, evaluating high fat diet induced hyperlipidemia for Amaranthus roxbhurgianus (L.) extract in Albino Wistar rats, Evaluating the Lipid profile

MATERIALS AND METHODS:

Collection, Identification and Extraction of Medicinal Plants:

The roots of Amaranthus roxburghianus collected in the month of febravery, 2021 from chittur dist. The plant materials were identified and authenticated by Prof. Madhav Shetty, Dept. of botany, Taxonomist, SV University, Tirupati. A voucher was kept in the Department of Pharmacognosy for reference.

Collected plant roots were shade dried for 15 days and they were coarsely powdered using a pulverizer. The pulverized plant materials were taken up for extraction using hydro alcohol in the proportion of 5:95. The extraction was carried out by cold percolation method. The extracts were then dried in vacuum and they were stored in desiccator and subsequently to a refrigerator.

PRELIMINARY PHYTOCHEMICAL TESTS:

Preliminary phytochemical tests were done for Ethanolic extracts of whole plant of *Amaranthus* roxbhurgianus(L) was subjected to qualitative tests for the identification of various active constituents. **Test for flavanoids**

Add a few drops of concentrated HCL and Mg turning to 1 ml of ethanol extract. Appearance of pink or magenta-red colour indicates the presence of flavanoids.

Test for cholesterol

To 2 ml of the extract 2 ml of the chloroform was added in a dry test tube. Then 10 drop of acetic anhydride and 2 to 3 drops of con. H2SO4 was added. A red colour changed to blue green colour.

Test for Alkaloids

To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendorff reagent. Any organic precipitate indicated the presence of alkaloids in the sample.

Test for terpenoids:

5ml of each extract was added to 2ml of chloroform and 3ml of con.H2SO4 to form a monolayer of reddish-brown coloration of the interface was showed to form positive result for the terpenoids.

Test for cardiac glycoside:

5mlof each extract was treated with 2ml of glacial

acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of con.H2SO4. A brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas acid layer, a greenish ring might form just gradually throughout thin Layer.

Test for steroids:

2 ml of acetic anhydride was added to 0.5 g of ethanolic extract of each sample with 2ml of H2SO4. The colour change from violet to blue or green indicated the presence of steroids

Test for Saponins:

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Acute toxicity studies:

Healthy albino rats of either sex of 2-2¹/₂-months-old of body weight 125-150 g were housed in polypropylene cages at 25±2°C with light dark cycle of 12 h in the Animal House of the study center are to be used for the study. It should be acclimatized for seven days. All animals are to be given with standard rat feed and water ad libitum. The experiments were performed after approval of the protocol by the minute of Institutional Animal Ethics Committee (IAEC) CPCSEA and animal care was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Development of cholesterol diet model:

High cholesterol diet consisting of Cholesterol (1%), sodium cholate (0.5%), sucrose(30%), casein (10%), butter (5%) and standard chow diet (53.5%) ad libitum, respectively, for the initial period of 2 weeks. The composition and preparation of HCD as were described elsewhere. [52]

Experimental design [52] High Cholesterol (HC)-Diet:

Wistar rats weighing 200- 250 gm, were divided into5 groups of 6 animals each. The animals of all the groups except normal group were given a lipid diet consisting of 2% cholesterol, 1% cholic acid and 2 ml coconut oil with standard pellet diet for 30 days. The first group (Normal control) received normal saline orally for 30days. The second group (High cholesterol diet (HCD)- positive control) was given High cholesterol diet (HCD) while the third Group recivied Atorvastatin 10 mg/kg, fourth and fifth groups were treated with hydroalcoholic extract of EEAB (2100 mg/kg and 200mg/kg, p.o.) respectively, once a day for 30 days. The fifth group was treated with Atorvastatin suspension prepared with 0.5% CMC (10mg/kg; p.o.), once a day for 30 days. After 30 days blood was collected by tail vein methods .. The collected samples were centrifuged for 10 minutes at 2000 r.p.m. and serum samples so collected were used for various biochemical tests.

Group 1: Normal control.1% CMC

Group 2: Hyperlipidemic control (Vehicle 1 ml/100g/day p.o).

Group 3: Hyperlipidemic treated with Atorvastatin (10 mg/kg, b.w./day p.o). Group 4: Hyperlipidemic treated with EEAB (100 mg/kg, b.w./day p.o).

Group 5: Hyperlipidemic treated with EEAB (200 mg/kg, b.w./day p.o).

PARAMETERS:

Serum Triglycerides (TG), total cholesterol (TC), and HDL-cholesterol (HDL-C) were estimated according to the methods of Zlatkis *et al.*,⁵³ Foster and Dunn ⁵⁴ and Burstein *et al.*,⁵⁵ respectively. The serum levels of VLDL and LDL cholesterol were calculated using Friedewald formula.⁵⁶ The atherogenic index (AI) was calculated by using the following formula.⁵⁷

Atherogenic index = $\frac{TC - HDL - C}{HDL - C}$

STATISTICS:

The values are expressed in mean \pm SEM. One way

ANOVA followed by Tukey's multiple comparison Test was used to analyse the effect of different doses of drugs when compared to control, with the help of Graph Pad Instat software, version 3.01. P<0.05 is considered as significant.

RESULTS:

Preliminary phytochemical screenig:

The results of the physiochemical, elemental analysis and quantitative estimation of phyto constituents followed by the pharmacological screening of various activities have been presented and discussed here below. In this study ethanolic extract of *Amaranthus roxbhurgianus*- showed positive to following phytochemical constituent's Carbohydrates, Glycosides, Flavonoids, Proteins, Sterols. The results are tabulated in Table 1.

Parameters	value
1. Alkaloid	-
2. Carbohydrates	+
3. Glycosides	+
4. Flavonoids	+
5. Tannins & Phenolic compounds	-
6. Proteins	+
7. Saponins	-
8. Sterols or Triterpenes	+

ACUTE TOXICITY STUDIES:

In acute toxicity study, no death was recorded, in the 14 days of observation period in the male and female animals given 2 g/kg of the ethanolic extract of *Amaranthus roxbhurgianus* orally. The animals did not show any changes in the general appearance during the observation period. Based on the results, the dose was fixed by oral route for further studies.

Effect of *Amaranthus roxbhurgianus* extract on cholesterol level of rats:

The hyperlipdemia control group showed significant (P<0.001) increase in cholesterol level when compared to normal control group. In hyperlipdemia control group mixed with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/ kg) showed significant (P<0.001) decrease when compared to hyperlipdemia control group. The cholesterol level of EEAB displayed highly appreciated decrease (P<0.001) when the values are looked upon the values of Atorvastatin (10mg/kg) (Table no.2, Figure: 1).

S.No.	Treatment	Cholesterol (mg/dl)	
1	Normal Control	142.4 ± 2.0	
2	HCD	209.0 ± 9.0 ###	
3	ATORVAS 10mg/kg	146.6 ± 5.2 **	
4	EEAB 100 mg/ kg	169.86±0.98 ^{##} ***	
5	EEAB 200 mg/ kg	155.3±0.94 ***	
	HCD HCD HCD HCD HCD HCD HCD HCD		

Table No 2: Effect of drugs on serum total cholesterol (mg/dl)	Table No 2: H	Effect of drugs	on serum total	cholesterol	(mg/dl)
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Effect of *Amaranthus roxbhurgianus* extract on cholesterol level of rats:

The values are expressed as mean ± SEM; ##P<0.01, ###P<0.001 when compared to normalcontrol group. ***P<0.001 when compared to HCD control group. (one way ANOVA followed by Tukey's multiple comparison Test)

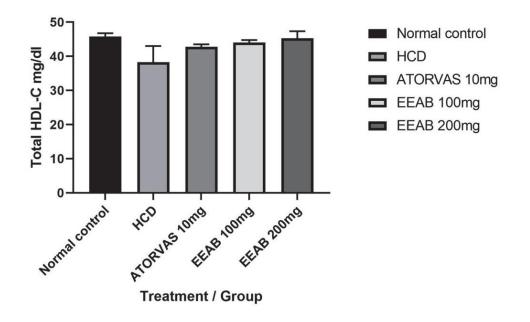
Effect of *Amaranthus roxbhurgianus* extract on HDL-C level of rats:

The HDL-C levels of hyperlipidemia control group provides the reported values of the lowest order having the significant (P<0.001) decrease when those values of extract brought in comparison to normal control group. In hyperlipidemia control group stirred with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/ kg) a prominent (P<0.05 and P<0.001) increase when it related to Atorvastatin treated group (Table no.3, Figure: 2).

S.No.	Treatment	HDL-C (mg/dl)
1	Normal Control	45.8 ± 0.991
2	HCD	38.31 ± 4.712 ^{##}
3	ATORVAS 10mg/kg	42.76 ± 0.745 ***
4	EEAB 100 mg/ kg	$44.01 \pm 0.761^*$
5	EEAB 200 mg/ kg	45.33 ± 2.009 ***

Table No 3: Effect of drugs on serum HDL-C level (mg/dl)

Figure: 2 Evaluation of serum HDL-C level



Effect of drugs on serum HDL-C level. n=6; the values are expressed as mean \pm SEM; ^{##}P<0.01, ^{###}P<0.001, when compared to normal control group. ^{*}P<0.05, ^{***}P<0.001 when compared to hyperlipidemia control group,

P<0.05, $^{P}<0.001$ when compared to Atorvastatin 10 mg/kg. (one way ANOVA followed by Tukey's multiple comparison Test).

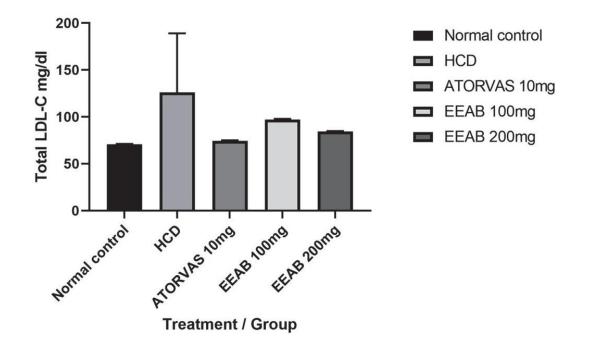
Effect of Amaranthus *roxbhurgianus* extract on LDL-C level of rats

The HDL-C levels of hyperlipidemia control group provides the reported values of the lowestorder having the significant (P<0.001) decrease when those values of extract brought in comparison to normal control group. In hyperlipidemia control group stirred with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/kg) a prominent (P<0.05 and P<0.001) increase when it related to Atorvastatin treated group (Table no.4, Figure: 3).

Table No 4: Effect of drugs on serum LDL-C level (mg/dl)

S.No.	Treatment	LDL-C (mg/dl)
1	Normal Control	70.9±0.27
2	HCD	126.±0.63##
3	ATORVAS 10mg/kg	74.59±0.56***
4	EEAB 100 mg/ kg	97.05±0.82*
5	EEAB 200 mg/ kg	84.51±0.37***

Figure: 3 Evaluation of serum LDL-C level



Effect of drugs on serum LDL-C level. n=6; the values are expressed as mean \pm SEM; ^{##}P<0.01, ^{###}P<0.001 ,when compared to normal control group. *P<0.05, ***P<0.001 when compared to

hyperlipidemia control group, P<0.05, ^P<0.001 when compared to Atorvastatin 10 mg/kg. (one way ANOVA followed by Tukey's multiple comparison Test).

Effect of Amaranthus *roxbhurgianus* extract on triglyceride level of rats.

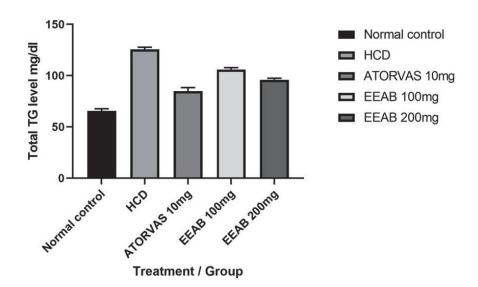
The triglyceride levels of hyperlipidemia control group provide the reported values of the lowest order having the significant (P<0.001) decrease when those values of extract brought in

comparison to normal control group. In hyperlipidemia control group stirred with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/ kg) a prominent (P<0.05 and P<0.001) increase when it related to Atorvastatin treated group (Table no.5, Figure: 4).

Table No 5: Effect of drugs on serum TG level (mg/dl)

S.No.	Treatment	TG (mg/dl)
1	Normal Control	65.58±2.11
2	HCD	125.58±2.11##
3	ATORVAS 10mg/kg	84.97±3.39***
4	EEAB 100 mg/ kg	105.78±2.08*
5	EEAB 200 mg/ kg	95.97±1.43***

Figure: 4 Evaluation of serum TG level



Effect of drugs on serum TG -level. n=6; the values are expressed as mean \pm SEM; ^{##}P<0.01, ^{###}P<0.001, when compared to normal control group. *P<0.05, ***P<0.001 when compared to hyperlipidemia control group, P<0.05, ^P<0.001 when compared to Atorvastatin 10 mg/kg. (one way ANOVA followed by Tukey's multiple comparison Test).

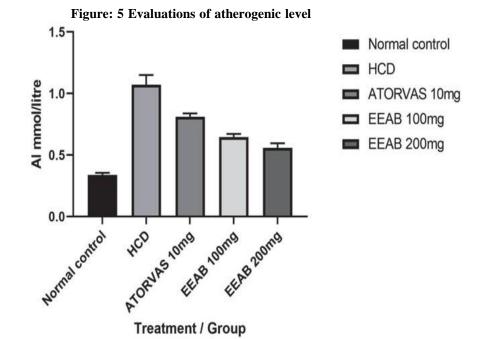
Effect of *Amaranthus roxbhurgianus* extract on Atherogenic Index of rats.

The Atherogenic index of hyperlipidemia control group pointed out a marked rise (P<0.001) increase when the same is brought in comparison to normal control group. In hyperlipidemia control group treated with Atorvastatin (10mg/kg); EEAB (100 and 200mg/kg) pointed out to a significant decrease (P<0.001) when related to hyperlipidemia control group. The atherogenic index of EEAB (100 mg) reflected

upon a significant (P<0.001) lowering in vales when compared with Atorvastatin 10 mg/kg (Table no.6, Figure: 5).

Table No 6: Effect of drugs on serum Atherogenic Index (mg/dl)

S.No.	Treatment	AI (mg/dl)
1	Normal Control	0.338 ± 0.0176
2	HCD	1.0721 ± 0.0784 ^{###}
3	ATORVAS 10mg/kg	$0.812 \pm 0.0259^{***}$
4	EEAB 100 mg/ kg	$0.647 \pm 0.025^{\#\#**}$
5	EEAB 200 mg/ kg	$0.559 \pm 0.036^{***}$



Atherogenic Index (AI) = log (TG/HDL-C)

The values are expressed as mean \pm SEM; ##P<0.01, ###P<0.001 when compared to normal control group. ***P<0.001 when compared to hyperlipidemia control. ^^^P<0.001, v^SP<0.05, when compared to Atorvastatin 20 mg/kg.(one way ANOVA followed by Tukey's multiple comparison Test).

DISCUSSION:

Preliminary phytochemical screening of ethanolic extracts of root of Amaranthus roxbhurgianus

were done. Result showed the presence of the following phytochemical constituents such as Carbohydrates, Glycosides, Flavonoids, Proteins, Sterols . (table : 3). The acute toxicity study does not show any deviation from the normal behavior. It is found that he extract is non- toxic and well tolerated. From this, LD 50 is determine from that the effective oral dose for the antihyperlipidemic study was selected. So, the present study was aimed to evaluate the antihyperlipidemic activity of the Amaranthus roxbhurgianus extract on HCD induced

hyperlipidemia in wistar rats. The Atorvastatin at a dose level of 10mg/kg was used as thestandard drug.

Hyperlipidemia was characterized by elevated serum total cholesterol (TC), low density (LDL), very low density lipoprotein(VLDL) and decrease in high density lipoprotein(HDL). These factors are exhibited to be the risk factors for coronary diseases. Hyperlipidemia contributes heart significantly in the manifestation and other development of a therosclerosis and coronary heart diseases (CHD). Cardiovascular diseases, including atherosclerosis, are the most common cause of mortality and morbidity worldwide.⁶¹ The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease.

Currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function Medicinal plants are used for various research purposes. Any herbal treatment for hypercholesterolemia has almost no side effects and, is relatively cheap, and locally available. They are effective in reducing the lipid levels in the system.

The in vivo antihyperlipidemic activity was evaluated on high cholesterol diet induced hyperlipidemic rats. high cholesterol diet, was used successfully to induced hyperlipidemia in previous studies. It causes these effects by activating HMG- CoA and inhibiting lipoprotein lipase activity). high cholesterol diet has been utilized in the hyperlipidemic model due to its convenience, reproducibility, and lack of undesirable underlying pathological conditions

The significant reduction in serum cholesterol,TG,LDL, ,AI, and also increased in total HDL level in the different dose level(100,200 mg/kg) in high cholesterol diet.

induced hyperlipidemia in rats Extact of *Amaranthus roxbhurgianus* showed (P.>;0.001) significant reduction in high cholesterol diet induced hyperlipidemia was compared with normal standarddrug Atorvastatin 10mg/kg.

The hyperlipdemia control group maintained a significant (P<0.001) raise in cholesterol level when compared to normal control group. Inhyperlipdemia control group treated with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/ kg) displayed a significant (P<0.001) decrease when compared to hyperlipdemia control group. This indicates that ethanolic extract of *Amaranthus roxbhurgianus* (EEAB) significantly reduces the serum cholesterol level.

HDL is considered to be a beneficial lipoprotein as it is involved in reverse cholesterol transport and has an inhibitory effect in the pathogenesis of atherosclerosis. So increased HDL-C has a cardio protective effect. The HDL-C levels decrease in hyperlipidemia control group and significantly increase (P<0.05 and P<0.001) in Atorvastatin (10mg/kg); EEAB *Amaranthus roxbhurgianus* (100, 200 mg/ kg).

LDL carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. A rise in LDL may cause deposition of cholesterol in the arteries and aorta, a risk factor for CHD.Our study points out that there is a significant (P<0.001) increase in LDL-C level of hyperlipidemia control by comparing with normal control group and LDL-C was found to get reduced significantly (P<0.001) in Atorvastatin (10mg/kg); EEAB *Amaranthus roxbhurgianus* (100, 200 mg/ kg).

The serum triglyceride levels have been reported to be an important risk factor as it influences lipid deposition and clotting mechanisms.Like cholesterol, it tends to damage vascular endothelial cells.High fat diet produces an increase in TG levels due to lipoprotein lipase triacylglycerol hydrolysis.TheTriglyceride level enables a significant (P<0.001) increase in hyperlipidemia control group on comparison to normal control group. In hyperlipidemia control group blended with Atorvastatin (10mg/kg); Amaranthus roxbhurgianus (100, 200 mg/ kg) produced a significant (P<0.001) decrease when compared to hyperlipidemia control group.TGlevel of EEAB100mg, appeared to have a significant decrease when compared to Atorvastatin

treated group.

Atherogenic index (AI) are predictors of coronary risk. The atherogenic index (AI) of plasma is a mathematical relationship between TG and HDL-C. The AI ranges from -0.3 to 0.1 (low), 0.1 to 0.24 (medium) and above 0.24 (high) risk of CV.The Atherogenic index of hyperlipidemia control group displayed a significant (P<0.001) increase once it is referred to normal control group. In hyperlipidemia control group treated with Atorvastatin (10mg/kg); *Amaranthus roxbhurgianus* (100, 200 mg/ kg) registered significant decrease (P<0.001) when compared to hyperlipidemia control group. The atherogenic index ofEEAB(100 mg) presented a significant (P<0.001) decrease when related to Atorvastatin 10 mg/kg.

EEAB also decreased atherogenic index which are Atherogenic coefficient, Cardiac risk ratio and Atherosclerosis index. Atherogenic index are powerful indicators of the risk of heartdisease: In this study, we observed that the ERCP significantly reduced atherogenic index. According to (Ikewuch et al) lower atherogenic index is protective against coronary heartdisease. Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and depletion in the tissue GSH levels.

CONCLUSION:

Plant materials are used throughout the developed and developing world as home remedies, in over-thecounter drug products, and as raw material for the pharmaceutical industry, and they represent a substantial proportion of the global drug market. Certain herbs have become popular over the years. but the public, medical practitioners and the media still have a poor understanding of herbal medicine. Evidence is emerging on the dangers of herbs. As in most situations, the truth lies hidden under the media hype, poorly understood science, and exaggerated claims. Lack of experience, information, and education about herbs make consumers, physicians, and other orthodox health care provider's easy victims of market exploitation and herbal myths. There is no rational reason behind the tendency to equate "natural" with "harmlessness."

The fact that something is natural does not necessarily make it safe or effective. In addition, a lack of knowledge of phytochemistry leads to misinterpretation and misunderstanding. It is very likely that some herbs will have side effects, interact with other medications, and be toxic. Information on isolated constituents should not be applied directly to the whole herb and studies on in vitro forms should not be confused with oral administration which was established by pharmacological screenings. In current scenario, herbs are the potent sources of medicines used in the treatment of various disease and disorders. Since, plants are used as medicine there is prompt need of evaluation of plant species, therefore, the present work was conceived to evaluate the phytochemical and pharmacological screening of few Indian medicinal plants.

Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyperlipidemic activity in atherogenic diet induced hyperlipidemic rats and led to the development of new Herbal formulation possessing antihyperlipidemic andantiatherosclerotic activities.

Hence it is going to be concluded that the potential benefits of the extracts of *Amaranthus roxbhurgianus* has been demonstrated well in advance and can be used further to demonstrate the antihyperlipidemic as well as controlling of both triglyceride levels and reducing the risk of factors of cholesterol inducers. The aforementioned results of the research suggest that the *Amaranthus roxbhurgianus* found to have the potential antihyperlipidemic action.

The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

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