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EXPLORING THE POTENTIAL EFFECTS OF *AMARANTHUS TRICOLOR* LEAVES IN DYSLIPIDEMIA AND DYSLIPIDEMIA INDUCED COMPLICATIONS IN RATS

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Keywords:

Amaranthus tricolor, The hypolipidemic effect, Cholesterol, Serum level, Atherogenic diet

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ABSTRACT: The present research work was considered to achieve preliminary phytochemical screening, acute oral toxicity, antioxidant activity, and to evaluate the antihyperlipidemic property of the aqueous and ethanolic extracts of leaves of *Amaranthus tricolor*. Antioxidant activities of the aqueous and ethanolic extracts of leaves of *Amaranthus tricolor* were investigated by Free Radical Scavenging Activity (DPPH method) and antihyperlipidemic activity by *in-vivo* effects on an atherogenic diet (2% cholesterol, 1% choline chloride and 2% Lard) induced rats hyperlipidemia. Our results exposed that *Amaranthus tricolor* showed potent antioxidant properties. In the cholesterol-induced hyperlipidemic model, groups of rats treated with extracts *A. tricolor* showed a significant reduction in total cholesterol (TC), triglycerides (TG) and in levels of SGOT, SGPT, ALP, LDH, and CKMB activities whereas increase in the level of high-density lipoprotein (HDL) compared to cholesterol-induced hyperlipidemic control group at dose-dependent manner but the effect was less than the standard drug Atorvastatin. It was observed from the histopathological findings that rats fed with *A. tricolor* extracts showed a decrease in granular degeneration caused by cholesterol feedings.

INTRODUCTION: Coronary artery disease (CAD) is one of the most important causes of death all over the world. Hyperlipidemia is one of the risk factors for CAD [1]. According to WHO, blood cholesterol contributes to about 56% of cases of cardiovascular diseases worldwide and causes about 4.4 million deaths every year. Of which the highest incidence being seen in Indian (5.6%) population followed by Chinese (4.7%) & Malaysians (3.6%).

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The National surveillance survey conducted reported that: Males (5%) have a higher prevalence of high blood cholesterol compared with females (4.3%) as reported by the WHO the annual number of deaths due to cardiovascular disease will rise from 17 million in 2008 to 25 million in 2030.

The larger number of NCD (non-communicable disease) death is caused due to cardiovascular diseases [2] (48%). Dyslipidaemia, which can range from hypercholesterolemia to hyperlipoproteinemia, is one of the many modifiable risk factors for coronary artery disease (CAD), stroke, and peripheral vascular disease. Raised serum lipids like, particularly of cholesterol along with the generation of reactive oxygen species (ROS), play a key role in the development of CAD and atherosclerosis [3, 4, 5].

As none among the available agents fulfill requirements of the desired drug, there is a need to explore the possibility of introducing effective, safe, and inexpensive alternatives [3, 4, 5] although currently available anti-dyslipidaemic agents include statins, fibrates, nicotinic acids, and bile acid sequestrants [3].

Since these drugs are not only costly but also have potential side effects, a search for alternative therapeutic agents was necessitated. Several synthetic hypocholesterolemic agents such as statins, fibrates, resins, and nicotinic acid are capable of efficiently reducing total plasma cholesterol (TC) levels, but LDL does not undergo any significant alteration. Also, synthetic hypolipidemic agents have side effects, *i.e.,* Liver failure, myopathy, rhabdomyolysis, renal insufficiency, intense cutaneous flush (accompanied by an uncomfortable feeling of warmth and pruritus nausea abdominal pain and are unable to increase HDL levels [6].

Phytotherapies are now recognized as several alternative countries for the treatment of CVS diseases, hyperlipidemia and the prevention of atherosclerosis, a huge number of plants have been found to lower plasma lipid levels [7, 8]. Few specific examples of the plants explored their anti-hyperlipidemic activity *Morus* [9], *Dioscoreophyllum cumminsii* [10], *Campomanesia adamantium* [11], *Cassia occidentalis* [12], *Curatella americana* [13], *Morus nigra* [14], *Labisia pumila* [15]. As several plants with various metabolites have the potential for therapeutic applications [6, 7]. *Amaranthus tricolor* L. belongs to the family *Amaranthaceae*. Preliminary phytochemical screening of the leaf extracts revealed the existence of steroids, triterpenoids, alkaloids, carbohydrates, proteins, saponins, flavonoids, tannins, and glycosides. The leaves are very nutritious. The nutrients existing in the leaves are carbohydrates, protein, vitamin A, vitamin C, riboflavin (Vitamin B_2), thiamin (Vitamin B_1) niacin, and minerals like calcium and iron. The plant has been scientifically reported to possess hepatoprotective (Simran *et al*., 2013), anti-inflammatory activities (Gopal *et al*., 2013), anti-cancer effects, anti-arthritic and cytotoxic activity, antioxidant (Samsul *et al*., 2013) activity. *A. tricolor* L. is one of the traditional medicines used in many folk claims, and the plant has been extensively used in Ayurveda and Siddha for treating menorrhagia, diarrhea, dysentery, hemorrhagic colitis, bowel hemorrhages, cough, and bronchitis. It is also used externally as an emollient poultice or a mouth wash to treat ulcerated conditions of the throat and mouth.

The leaves of *A. tricolor* have been used against external inflammations, as a diuretic, and as a treatment for bladder distress). High-fat diet-induced hyperlipidemia in rats as characterized by decreased levels of antioxidant enzymes, increased levels of cholesterol profile, and damages in hepatic tissues. However, long-term consumption of a high-fat diet that makes lipid changes could increase the possibility of metabolic function damage. Hence, considering the antioxidant, anti-inflammatory the present study was designed to study the effect of *Amaranthus tricolor* for antioxidant and hypolipidemic activity in cholesterol diet-induced hyperlipidemia in rats [15-19].

MATERIALS AND METHODS:

Collection and Identification of the Plant Materials: *Amaranthus tricolor* leaves were collected. A voucher specimen (Voucher no. 1289) was kept at the Department of Botany, Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh, India after identification of the plant by Dr. K. Madhava Chetty, Assistant Professor, Botany Department.

Extraction of Plant Material: The collected roots were dried in the shade and grind with a mechanical grinder. About 30g powder poured in separating funnel with 50% methanol for 48 h. The collected residues were kept at 55-60 °C in a water bath to concentrate it and finally transfer into the Hot Air Oven to dry it. The about 5.8g crude extract was prepared (Yield= 19%) and used for further studies [20].

Preliminary Phytochemical Screening: Standard screening test were carried out for various plant constituents. The methanolic and aqueous crude extract was screened for presence or absence of secondary metabolites such as alkaloids, tannins, steroids, phenols, flavonoids, saponins, and phlobatannins, *etc.* using standard procedures to identify the constituents as described by Khandelwal [20] (2005).

Experimental Animals: Male Wistar rats weighing 200-250 g were acclimatized to the experimental room having temperature 23±2°C, controlled humidity conditions, and 12:12 h light and dark cycle. Animals were caged in polypropylene cages in a group with a maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (IAEC), Resolution no-2015/837/ac/PhD/06, IFTM University Lodhipur Rajput, Delhi Road, Moradabad, India.

Acute Toxicity Study: Acute toxicity was determined according to the OECD guidelines No. 423. Female albino rats (n = 3 per step) were selected by random sampling technique. The rats were kept fasting for overnight providing with water *ad libitum*. The aqueous and ethanol extract of *Amaranthus tricolor* leaves were administered separately to two sets of rats (n = 3 per set) at a dose of 300 mg/kg by intra-gastric tube.

Food was withheld for further 3 - 4 h and observed once in every 30 min during the first 24 h and daily after that, for 14 days for any mortality. If mortality was not observed for any animal, then the procedure was repeated with higher doses such as 1000 and 2000 mg/kg, and the animals were observed for toxic symptoms as per the above procedure [21].

Experimental Induction of Atherosclerosis: In rats, hyperlipidemia was induced by daily administration of 1% choline chloride, 2% cholesterol, and 2% lard over 21 days. The cholesterol diet was given at approximately the same time every day [22, 23].

Preparation of Feed: Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then ground into fine powder in a mixer grinder. The other ingredients, *i.e.* 1% choline chloride, 2% cholesterol and 2% lard too, were added in the mixer grinder in ascending order of their quantity and mixed well. This dried powder was then mixed with the same quantity of water every time to make small balls of feed, and later this was stored in self-sealing plastic covers in the refrigerator at 2°C to 8°C.

The feed for the normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other excipients. This preparation of feed was done once in three days for all the animals [22, 23].

Preparation of Herbal Drug Extract and Standard Drug Atorvastatin: Different doses of EEAT, AEAT (200 mg/kg and 400 mg/kg) and Atorvastatin (10 mg/kg) were dissolved in 2% tween 80 to obtain a concentration required [24].

Protocol for Anti-hyperlipidemic Activity:

TABLE 1: THE EXPERIMENTAL ANIMALS WERE DIVIDED INTO SEVEN GROUPS, EIGHT ANIMALS IN EACH GROUP

S. no.	Group no. (N)	Description	Animal per Group (n)
1	I	Normal control: normal diet	8 Rats
2	II	Positive control: High cholesterol diet	8 Rats
3	III	Standard Drug: high cholesterol diet + (Atorvastatin 10 mg/kg)	8 Rats
4	IV	(Ethanolic extract of *Amaranthus tricolor)* (200 mg/kg)	8 Rats
5	V	(Ethanolic extract of *Amaranthus tricolor)* (400 mg/kg)	8 Rats
6	VI	(Aqueous extract of *Amaranthus tricolor)* (200 mg/kg)	8 Rats
7	VII	(Aqueous extract of *Amaranthus tricolor*) (400 mg/kg)	8 Rats

Blood Sample Collection and Analysis: On day 22nd, animals were anesthetized with diethyl ether, and blood was collected by a retro orbital puncture. The blood was allowed to clot for 30 min at room temperature and then was subjected to centrifugation at 2000 rpm for 15 min to obtain serum [22]. The resulting upper serum layer was collected in clean, dry, and labeled micro-centrifuge tubes. This serum was analyzed for serum total serum cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) was estimated using diagnostic kits. High-density lipoprotein ratio (HDL-C ratio). Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) activities, creatine kinase-MB (CKMB) were also measured by using diagnostic kits.

Histopathological Analysis: Histopathological study was performed in all the groups of animals. The liver and heart sections were isolated and preserved in 10% formalin [25]. The liver and heart sections were then evaluated for any architectural change.

Statistical Analysis: The results were expressed as mean ± S.E.M (n=8). The statistical analysis involving seven groups was performed by means analysis of variance (ANOVA) followed by Dunnett test. p-value at <0.05 was considered as statistically significant. Data were processed with graph pad prism version 5.00 software.

RESULTS:

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE ETHANOLIC AND AQUEOUS EXTRACT OF LEAVES *AMARANTHUS TRICOLOR*

S. no.	Solvent→ Phytochemical↓	Methanol	Water
1.	Carbohydrates	+	+
2	Proteins	+	+
3	Aminoacids	+	+
4	Steroids	+	+
5	Cardiac glycosides	+	+
6	Flavonoids	+	+
7	Alkaloids	+	+
8	Tannins	+	+

(+ Presence of constituent, - Absence of constituent**)**

TABLE 3: EFFECT OF AT ON ATHEROGENIC DIET & ATORVASTATIN ON SERUM LIPID LEVELS

	Normal control	AD Diet control	Atorvastatin 10 mg/kg	EEAT 200 mg/kg	EEAT 400 mg/kg	AEAT 200 mg/kg	AEAT 400 mg/kg
TC [f]	68.8±4.3***	144.9±7.7	73.3±6.8***	116.8±8.5***	102.9±15.9***	119.5±5.8***	103.7±13.3***
TG [f]	81.1±8.7***	174.9±4.5	103.7±4.9***	142.9±8.9***	138.3±9.7***	141.6±7.5***	119.4±3.5***
HDL [f]	39.6±3.2***	30.4±1.8	35.6±2.9**	31.9±3.5	32.8±3.32	27.4±2.8	33.8±1.2
Non HDL [f]	29.2±2.9***	114.5±8.3	37.7±5.5***	84.9±6.9***	70.1±14.9***	92.1±6.4***	69.9±2.5***
TC:HDL	2.35±0.1***	4.83±0.4	2.05±0.1***	3.66±2.42	3.13±0.5**	4.36±0.6	3.06±0.2**

Ƒ mg/dl, units Values are mean ± SEM (n=8)

Statistical Analysis: The results were expressed as mean ± S.E.M (n=8). The statistical analysis involving seven groups was performed by means analysis of variance (ANOVA) followed by Dunnett test. p-value at <0.05 was considered as statistically significant. p values: *<0.05, **<0.01, ***<0.001, as compared with AD diet, Data were processed with graph pad prism version 5.00 software.

Antioxidant Activities of *Amaranthus tricolor*: The radical scavenging activity of the plant extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2, 2-diphenyl-1- picrylhydrazyl) as described by Molyneux. 0.1 mM solution of DPPH in methanol was prepared, and 1.0 ml of this solution was added to 1.0 ml of extract solution in methanol at different concentrations (5 µg/ml - 500 µg/ml). After thirty minutes, the absorbance was measured at 517 nm, and ascorbic acid was used as a reference standard at concentrations ranging from 5-50 µg/ml.

The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of the test sample)/Absorbance control] × 100.

The antioxidant activity of the extracts was expressed as an inhibitory concentration (IC). IC_{50} is defined as the concentration in µg/ml of extracts sufficient to obtain 50% of a maximum scavenging capacity [11].

TABLE 4: PERCENTAGE INHIBITION AND IC_{50} VALUES OF DPPH RADICAL IN-VITRO BY ETHANOLIC EXTRACT, AQUEOUS EXTRACT OF *AMARANTHUS TRICOLOR* LEAVES AND ASCORBIC ACID

Extracts	50	100	200	500	1000	IC_{50}
EAT	25.6±1.44	37.0±0.82	46.6±0.59	61.2±1.74	71.8±0.48	207
AEAT	4.21±1.28	15.7±1.07	23.8±1.58	40.7±1.98	57.9±1.57	848
AA	37.06±0.89	47.40±0.69	66.22±0.72	81.77±1.74	93.04±2.07	104

Quantity in Micrograms (Mg/ml), Mean ±SEM: AT *A. tricolor*. L, EAT: Ethanolic extract of AT, AEAT: Aqueous extract of AT, AA: Ascorbic acid

Statistical analyses were achieved by one-way analysis of variance. The IC_{50} values were calculated by linear regression analysis. Results were calculated by employing the statistical software (Graph pad prism version 5.00 software.)

TABLE 5: EFFECT OF ATORVASTATIN 10 mg/kg, EEAT 200 mg/kg, EEAT400 mg/kg, AEAT 200 mg/kg, AEAT 400 MG (PO) ON HEPATIC AND CARDIAC ENZYMES IN RATS UNDER DYSLIPIDEMIA CONDITION

	Normal control	AD Diet control	Atorvastatin 10 mg/kg	EEAT 200 mg/kg	EEAT 400 mg/kg	AEAT 200 mg/kg	AEAT 400 mg/kg
SGOT	56.53±0.94***	85.58±1.54	42.25±1.72***	65.24±1.62***	58.32±1.85***	72.54±2.42***	60.34±1.42***
SGPT	51.1±1.87***	88.8±2.16	51.05±2.6***	76.82±1.62***	56.32±2.42***	78.32±1.85***	58.56±2.41***
ALP	96.3±1.62***	307.21±7.09	185.21±3.2***	226.32±3.81***	198.31±6.32***	232.41±5.2***	204.21±4.21***
LDH	276.7±45.0***	612.4±47.12	571±23.0***	582.12±4.28***	576.24±4.21***	588.14±3.21***	574.22±4.31***
CKMB	309.45±6.71***	358.7±4.81	330.41±5.38***	348.22±5.28***	335±4.62***	345±6.72***	322±4.58***

The results were expressed as mean ± S.E.M (n=8). The statistical analysis involving seven groups was performed by means analysis of variance (ANOVA) followed by Dunnett test. P-value at < 0.05 was considered as statistically significant. P-values: *<0.05, **<0.01, ***<0.001, as compared with AD control Data were processed with graph pad prism version 5.00 software.

Effect of *Amaranthus Tricolor* Leaves Extract on Liver Histopathology of Cholesterol-Rich HFD Treated Rats: A liver section of normal rat liver showed no cellular degeneration and necrosis **Fig. 1A**. AD fed rat showing fatty granular degeneration with liver edema, altered hepatocyte architecture, and several fat globules were seen **Fig. 1B**.

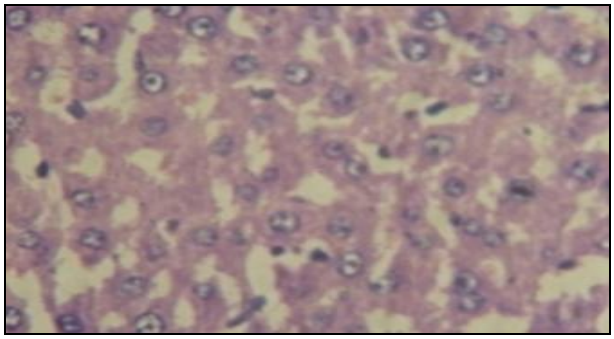

FIG. 1A: LIVER SECTION (40X) OF NORMAL FED DIET SHOWING RAT SHOWING NORMAL HEPATOCYTE ARCHITECTURE

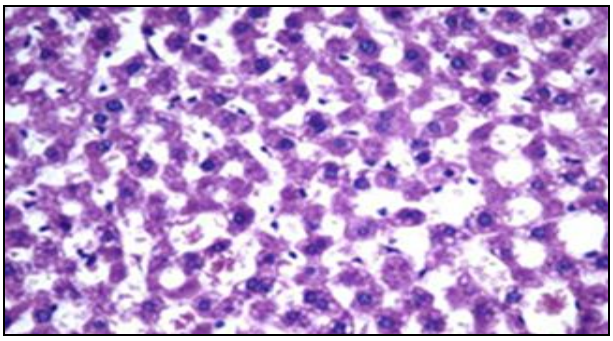

FIG. 1B: LIVER SECTION (40X) OF AD FED RAT SHOWING FATTY GRANULAR DEGENERATION WITH LIVER EDEMA

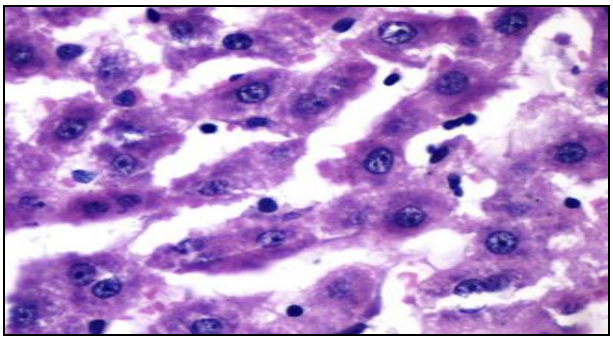

FIG. 1C: LIVER SECTION (40X) OF ATORVASTATIN TREATED RATS SHOWING NORMAL HEPATOCYTES

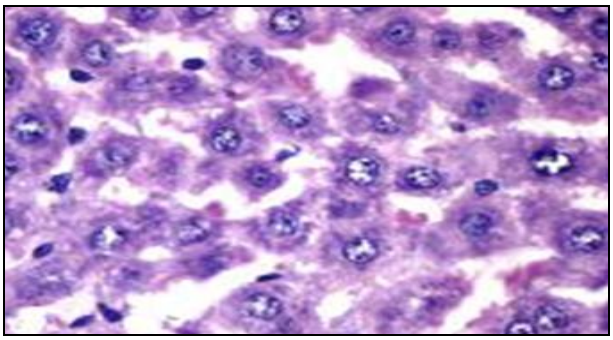

FIG. 1D: LIVER SECTION (40X) OF EEAT HIGH DOGE (400 mg/kg) TREATED RAT SHOWING NORMAL HEPATOCYTE ARCHITECTURE

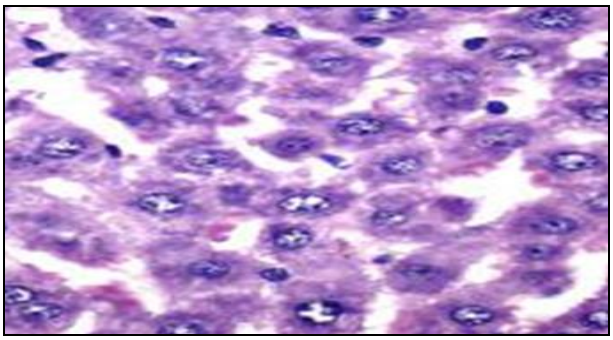

FIG. 1E: LIVER SECTION (40X) OF AEAT HIGH DOGE (400 mg/kg) TREATED RATS SHOWING NORMAL HEPATOCYTE ARCHITECTURE

But animals treated with Atorvastatin **Fig. 1C**, EEAT 400 mg/kg **Fig. 1D** and AEAT, 400 mg/kg **Fig. 1E** restored the normal architecture, and no fatty liver changes were seen.

Histopathology of Coronary Artery:

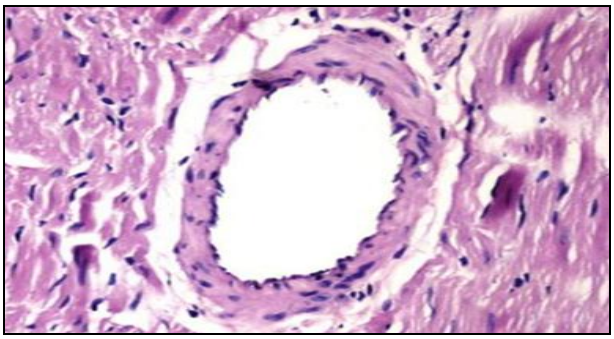

FIG. 2A: SECTION OF CORONARY ARTERY (20X) FOR NORMAL DIET FED RATS SHOWING NORMAL INTIMA

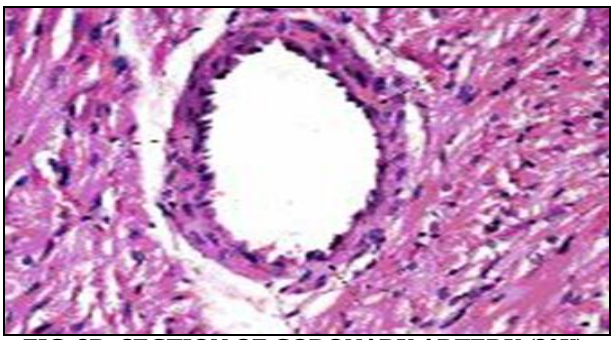

FIG. 2B: SECTION OF CORONARY ARTERY (20X) AD FED RAT SHOWING SWOLLEN ENDOTHELIAL CELL IN INTIMA WITHSPLITTING OF THE ELASTIC INTIMA

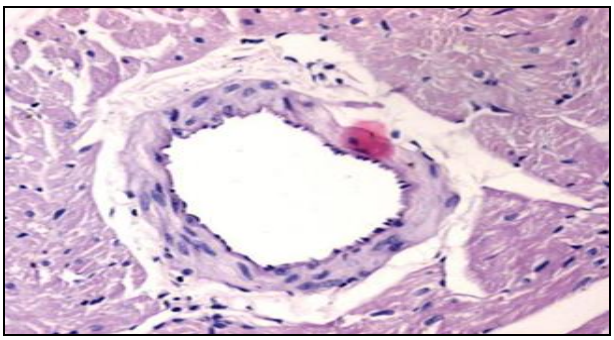

FIG. 2C: SECTION OF CORONARY ARTERY (20X) OF ATORVASTATIN TREATED RAT

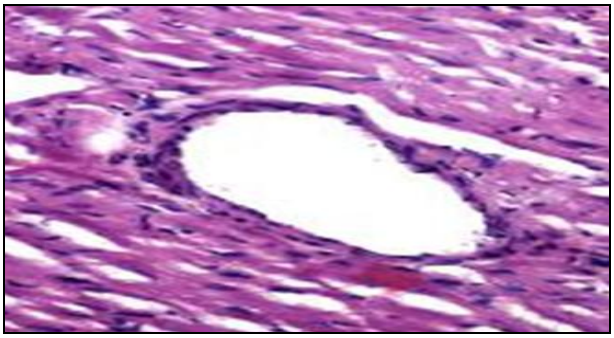

FIG. 2D: CORONARY ARTERY SECTION (20X) OF RAT TREATED WITH EETT 400 mg/kg SHOWING NORMAL INTIMA

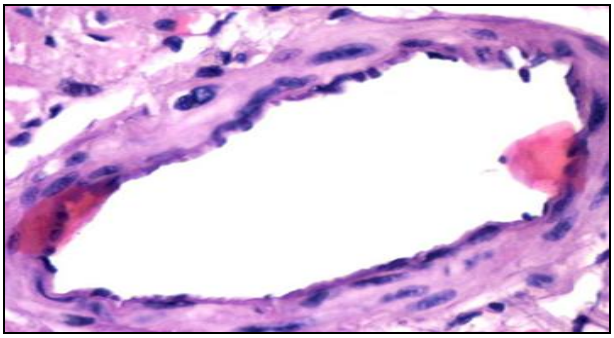

FIG. 2E: CORONARY ARTERY SECTION (20X) OF RAT TREATED WITH AEAT 400 mg/kg SHOWING FEW SWOLLEN ENDOTHELIAL CELLS

Sections of coronary artery studied in rats fed with AD indicated swollen endothelial cells in the intima of the coronary artery, and there was often a splitting of the elastica interna seen **Fig. 2B**. Whereas normal diet-fed rats did not show any of these changes, **Fig. 2A**. Atorvastatin 10 mg/kg of treated rats showed normal histology of coronary arteries without any changes. **Fig. 2C**, and **Fig. 2D**. Coronary artery of rat treated with *Amaranthus tricolor* showed a moderate number of swollen endothelial cells **Fig. 2E**.

DISCUSSION: Hyperlipidemia is a well-known risk factor for CVDs. Atherosclerotic CAD is one of the main causes of premature death worldwide, and it is expected to be the most important cause of mortality in India [29]. Statins are widely used to control hyperlipidemia, but these are not fully effective, completely safe, and free from adverse events. The complication with statins is liver failure, myopathy, rhabdomyolysis, harmful to the kidney, and often cause kidney damage. Additionally, statins may cause cardiomyopathy.

Headache, myalgia and dizziness, *etc* as well as Contraindicated during pregnancy, nursing mothers, children. Recent clinical trials showed that statin use had been linked to an increase in type 2 diabetes [30]. The leaves *Amaranthus tricolor* is traditional medicine. It has several useful pharmacological properties such as anti-inflammatory, antioxidative, hepatoprotective, anti-tumor, anti-ulcer activity, inhibitory effect on cobra venom effect, diuretic activity, *etc*. In the present study, we carried out experiments to investigate the antioxidant activity by free radical scavenging activity (DPPH method), and antidyslipidemic activity of ethanolic and aqueous extract of *Amaranthus tricolor* leaves in the high-fat diet (HFD) fed dyslipidemic rat model.

It has been well established that nutrition plays an important role in the etiology of hyperlipidemia and atherosclerosis [31]. The composition of the diet selected in this study has been based on various previous published research articles and a pilot study conducted for this study for hyperlipidemic effects. In our study, we chose the atherogenic diet (2% cholesterol, 1% choline chloride, and 2% Lard) [32, 22,] which contains the common ingredients as in our daily food. It has been reported that cholesterol alone does not significantly elevate serum cholesterol and TG levels therefore high levels of saturated fatty acids (SFA) for hypertriglyceridemia and cholic acid salts are essential for the development of hyper-cholesterolemia in a rat model of study as it amplifies the effect of dietary cholesterol [33, 34]. A diet containing SFA (Lard) increases the activity of HMG-CoA reductase, the rate-determining enzyme in cholesterol biosynthesis; this may be due to the higher availability of acetyl CoA, which stimulates the cholesterol genesis rate.

Moreover, this could be associated with a downregulation in LDL receptors by the cholesterol and SFA in the diet, which could also explain the elevation of serum LDL cholesterol levels or non-HDL levels either by changing hepatic LDL receptor activity, the LDL production rate or both. The activity of cholesteryl ester transfer protein (CETP), a key enzyme in reverse cholesterol transport and HDL metabolism increases in high-fat diet and mediates the transfer of cholesteryl esters from HDL cholesterol to TG rich particles in exchange for TG. This leads to increased plasma concentrations of TGs & decreased concentrations of HDL cholesterol [34]. It has been reported that a series of human illness such as cancer, atherosclerosis, cerebrovascular diseases, and cardiac diseases, can be linked to the damaging action of extremely reactive free radical. Ascorbic acid is an effective free radical scavenger, so when compared to such pure component, IC_{50} of ethanolic and aqueous extracts shows that *A. tricolor* is a potent free radical scavenger **Table 2**. Samsul *et al*., 2013 described that plants phenolics, in particular, phenolic acid, tannins, and flavonoids are recognized to be effective antioxidant and occur in leaves, roots, bark, nuts, seeds, fruits and vegetables.

In addition to their antioxidant effects, these compounds display a wide variety of pharmacological activities [11]. The data indicate that the antioxidant activity of leaf extracts of *A. tricolor* may be to be due to the presence of flavonoids like flavones, flavones, flavonols, and tannins. In a very recent year, potent free radical scavengers have attracted a tremendous attentiveness as possible therapeutic against free radical-mediated diseases. In the present study, the extracts of EEAT & AEAT revealed significant antihyperlipidemic activity in a cholesterol-induced hyperlipidemic model of rats. In the same manner as in the statin-treated group.

The extracts of *A. tricolor* induced an increase in serum HDL-C levels in the hyperlipidemic models but decrease in the serum TC, TG, non HDL, TC: HDL as shown in **Table 3**. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as “reverse cholesterol transport” hence increased serum HDL-C levels may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process. Rats treated with extracts of EEAT, AEAT at 200 and 400 mg/kg as described in protocol **Table 1** and Atorvastatin 10 mg/kg caused a significant decrease in the levels of SGOT, SGPT, ALP, CKMB activities **Table 5**. When compared to the cholesterol-induced hyperlipidemic control group. Histopathology of coronary arteries from AD fed animals showed swollen endothelial cells

in the intima with often a splitting seen in elastica interna. Atorvastatin 10 mg/kg, EEAT, AEAT at 400 mg/kg treated rats showed normal histology of coronary arteries **Fig. 2A** to **Fig. 2E**. Whereas in the histopathology of lever AD fed rat showing Granular fatty degeneration with liver edema. **Fig. 1B** but rat treated with EEAT, AEAT with 400 mg/kg shows normal hepatocyte architecture in the same manner as with Atorvastatin treated rat **Fig. 1C** to **Fig. 1E**.

CONCLUSION: By these results, it may be confirmed that the aqueous and ethanolic leaf extracts of *A. tricolor* have antioxidant activity. The present investigation has suggested the significant protection in lowering TC, TG, non-HDL, TC: HDL ratio, SGOT, SGPT, ALP, LDH, CKMB, and increasing HDL levels.

Histopathology study shows normal hepatocyte architecture by EEAT and AEAT at 200 mg/kg, and 400 mg/kg in the same manner as with Atorvastatin treated rat and Coronary artery section of rat treated with leaf extracts of A.T showing almost normal intima.

The results of blood sample analysis strongly mention that the significant hypolipidemic activity of this medicinal plant, and finally, histopathology study supports the same. However, there is a requirement for further research to work for more insight to the possible mechanisms.

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