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EVALUATION OF HYPOLIPIDEMIC AND ANTI-OBESITY ACTIVITIES OF *MOMORDICA DIOICA* ROXB. FRUIT EXTRACTS ON ATHEROGENIC DIET INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Cardiovascular diseases are leading cause of death and there is need for development of new therapeutics. Plant based therapeutics play an important role in the management of cardiovascular disease and considering this, the present study was designed to evaluate the antioxidant, hypolipidemic and anti-obesity effect of extracts of *Momordica dioica* Roxb. fruits (MDR). The crude methanolic extract (MEMD) was prepared by soxhlet extraction and fractionated into petroleum ether, chloroform (NFEMD) and ethyl acetate (FFMD). The marc was macerated with chloroform water to yield the aqueous extract (AEMD). All extracts were evaluated for their *in vitro* antioxidant and free radical scavenging activities and among the extracts; MEMD & FFMD have shown best antioxidant and free radical scavenging activities. MEMD and FFMD having best activities owing to their high phenolic and flavonoid content were selected for *in-vivo* studies on atherogenic diet (AD) induced hyperlipidemic rats. Atherogenic diet alone treated animals have developed hyperlipidemia and obesity when compared with normal animals. Whereas MEMD and FFMD significantly ($p \leq 0.05$) reduced the elevated levels of total cholesterol (TC), serum triglyceride (TG), LDL-cholesterol and VLDL-cholesterol, AST and ALT and elevate the decreased level of HDL-cholesterol. Other observations shows that rats treated with MEMD & FFMD underwent a time-dependent reduction in body weight, organ weight and increase in locomotors activity. These results suggest that, MDR extracts possess good hypolipidemic and anti-obesity activity, which may be due to its antioxidant and free radical scavenging potential. MEMD has more potential than FFMD because of high phenolic and flavonoid content.

Keywords: *Momordica dioica* Roxb, Anti-obesity, Atherogenic diet, Hypolipidemic activity.

INTRODUCTION

Hyperlipidemia is one of the greatest risk factors which further lead to coronary heart diseases, stroke, atherosclerosis and ischemic heart diseases, which are the primary cause of death. Hyperlipidemia associated with lipid disorders are considered to cause the atherosclerotic cardiovascular diseases.^{1,2} Obesity is another serious disease condition in recent years which result in various other serious diseases³. There are

millions of people suffering from obesity which further leads to cardiovascular diseases and less number of potential treatment are available for the treatment of these diseases. According to world health organization (WHO), more than half of the total mortalities are associated with cardiovascular diseases.⁴ It is estimated that 12 million deaths per year occur from cardiovascular diseases⁵, while one million of deaths in the

European country occur due to obesity per year. Since available hypolipidemic drugs have high side effects, while herbal treatment are safe and is relatively cheap and locally available⁶, around 80% of the world population is relied upon plant for their medication. Therefore the use of the medicinal plants is increasing in many countries where 35% of drugs contain natural products. Moreover; synthetic drugs are very expensive to develop. It is therefore essential that efforts should be made to evaluate new medicinal plants to develop cheaper drugs.⁷ *Momordica dioica* Roxb. (MDR) is a perennial climbing creeper belonging to the family Curcubitaceae and generally found in the forests of Southern India, Bengal, Maharashtra and Madhya Pradesh and occurs naturally throughout India, Sri Lanka, Burma, China and Malaya; cultivated in the Deccan.⁸ The plant is reported up to an altitude of 1500 m in Assam and Garo hills of Meghalaya. Kakrol is a Cucurbitaceous crop originated in the Indo-Malayan region.⁹ Alcoholic extract of MDR possess anti-allergic activity and found that it is effective to inhibit passive cutaneous anaphylaxis in mouse and rat.¹⁰ Shreedhara *et al.*¹¹ reported the anti-fertility activity of aqueous and ethanolic extract of root of MDR in female rat. Ilango *et al.*¹² observed the analgesic and anti-inflammatory activities in MDR fruit pulp hexane and methanol extracts. The plant is reported for anti-feedent activity and exhibited moderate and concentration dependent anti-feedent activity.¹³ The aqueous and ethanolic extracts of MDR have antioxidant and hepatoprotective activity. Among the extracts, ethanolic extract was found to be more potent hepatoprotective. The antioxidant and free radical scavenging activities were positive for both ethanolic and aqueous extract. This activity may be due to free radical scavenging and antioxidant activities which may be due to presence of flavonoids in extracts.¹⁴ The plant exhibited hypoglycaemic and hypolipidemic activities on alloxan-induced diabetic rats.¹⁵ Local people routinely use this fruit as vegetables and also for the treatment of various diseases. The hypolipidemic activity of MDR was reported earlier¹⁶ but no scientific investigation was

conducted on the particular phytochemical constituent which is responsible for hypolipidemic effect. As on today there is no reference for an anti-obesity activity of MDR. With this background the present study has been undertaken to evaluate the hypolipidemic and anti-obesity activity of various extract of MDR.

MATERIALS AND METHODS

Plant Material

The fresh fruits of MDR were collected in the month of July from Mysore local market and it was identified and authenticated by Dr. M. N. Naganandini, Asst. Professor, Dept. of Pharmacognosy, JSS College of Pharmacy, Mysore. The fruits were cleaned to remove impurities and cut into small pieces and shade dried. The fruit powder was weighed and stored in air tight containers. A specimen sample (SAMD032) is deposited in the Dept. of Pharmacognosy of JSS College of Pharmacy, Mysore.

Preparation of the Extracts

The dried powdered fruits of MDR were extracted with 100% methanol (analytical grade) using soxhlet extractor (MEMD). The marc obtained after methanolic extraction was macerated with chloroform water (5:95) for 3 days to get aqueous extract (AEMD). Some portion of MEMD was further fractionated with petroleum ether, chloroform, ethyl acetate and water depending upon solubility into flavonoid (FFMD) and non-flavonoid fractions (NFFMD). All the extracts were concentrated using flash rotary evaporator and water bath and then dried under vacuum.

Preliminary Phytochemical Screening

All the extracts were subjected to preliminary phytochemical studies to know the presence of various phytochemicals and their distribution in different fractions and extracts.¹⁷⁻²¹

In vitro antioxidant activity

All the extracts of MDR i.e. MEMD, FFMD, AEMD and NFFMD were subjected to different *in vitro* antioxidant and free radical scavenging activity like DPPH radical scavenging assay²², alkaline DMSO method²³, reducing power²⁴, hydrogen peroxide scavenging activity²⁵ and

ferrous sulphate stimulated lipid peroxidation.²⁶ All the experiments were performed in triplicate and EC₅₀ values were calculated by linear regression of the plot. Ascorbic acid was used as standard antioxidant in all the methods.

Animals

Albino rats of either sex weighing 160-200 g were used. Animals used in the study were procured from JSS Medical College, animal facility centre, Mysore. Animals were acclimatized to the experimental condition for one week prior to the experiment under controlled conditions of temperature (27 ± 2⁰C) and were housed in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of six animals in each cage. The rats were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee (Approval number-047/2010), JSS College of Pharmacy, Mysore, Karnataka.

Hypolipidemic & Anti-Obesity Activity of MDR Extracts^{2 & 27-28}

Experimental design

Forty two albino rats weighing 160-200 gm were randomly divided into seven groups of six each and kept in their cages for 1 week prior dosing to allow for acclimatization to the laboratory conditions. The chronic experimental hyperlipidemia and obesity was produced in rats by the following treatment as shown in table 1.

Selection of dosage

The dose of extracts of MDR fruit extract was arbitrarily chosen as 250 & 500 mg/kg. Dose of Atorvastatin was calculated based on a human dose of 10 mg per day. All preparations were suspended in 0.5% sodium CMC for oral administration.

Induction of hyperlipidemia and obesity

Atherogenic diet was used to induce hyperlipidemia in rat. Atherogenic diet is administered in rats by oral route. Rats were made hyperlipidemic by the oral administration (P.O) of Cholesterol (400 mg/kg) along with cholic acid (50 mg/kg) in coconut oil for 25 days, once daily.

Evaluation of hypolipidemic and anti-obesity activity

A) Body weight

The change in body weight (g) was recorded on 1st and then on 26th day of treatment period in each group animals.²⁸

B) Organ weights

The animals were sacrificed on 26th day by cervical dislocation and then different organs (kidney, liver, heart, spleen) were removed and weighed.²⁸

C) Locomotor activity

It was recorded on 26th day using open field behaviour test apparatus. The apparatus consisted of a circular wooden arena of 75 cm diameter and wall with a height of 25 cm. Open field test was performed by placing the rat in the center circle and recording the ambulatory activity, the frequency of rearing and grooming was recorded for a 5 min test period.²⁸

D) Biochemical parameters

On 26th day 2 ml of blood was collected by carotid bleeding while sacrificing the animal. The blood was allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 2500 rpm for 15 min. Serum was analyzed for serum TG, TC, HDL-C, LDL-C, serum AST and serum ALT levels using standard diagnostic kits using Merck auto-analyzer. The VLDL cholesterol levels were calculated using empirical equation of Friede Walds equation.²⁹

Statistical analysis

The values were expressed as Mean±Standard Error of Mean (SEM) of the indicated number of experiments/animals. All data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

Preliminary Phytochemical Analysis

The percentage yield of MEMD, AEMD, FFMD, NFFMD were found to be 14.85, 11.64, 8.67, 3.56 % w/w respectively. Preliminary phytochemical

analysis of various extracts revealed the presence of phytochemicals as shown below.

Extracts	Phytochemical Present
MEMD	Triterpenes, tannins, carbohydrates, reducing sugars, flavonoids, alkaloids, cardiac glycosides.
AEMD	Cardiac glycosides, flavonoids, tannins, reducing sugars, carbohydrates, saponins.
FFMD	Saponins, flavonoids, cardiac glycosides.
NFFMD	Triterpenes and alkaloid.

In Vitro Antioxidant Activity

MEMD, AEMD, FFMD and NFFMD exhibited antioxidant and free radical scavenging activity in graded concentrations. Results showed MEMD and FFMD as potent free radical scavengers and antioxidants among all the fractions and this activity is due to higher concentration of flavonoids and phenolic compounds in these. Since, MEMD and FFMD were found to be potent antioxidants they were selected for *in vivo* hypolipidemic activity.

DPPH Radical Scavenging Activity

The IC₅₀ values of MEMD, AEMD, FFMD & NFFMD for DPPH scavenging activity was found to be 249.82±17.87, 613.62±22.87, 335.52±17.65, 389.9±18.60 µg/ml respectively, whereas ascorbic acid used as a reference standard showed scavenging potential with an IC₅₀ value of 3.17±0.50 µg/ml as shown in Table 2. Among all extracts MEMD and FFMD exhibited better DPPH scavenging potential and was found to be dose dependent.

H₂O₂ Scavenging Activity

Generally, both AQMD & NFFMD possess less activity, whereas MEMD & FFMD being more effective (IC₅₀- 293.80±12.87 and 367.78±22.8 µg/ml) than AQMD & NFFMD (IC₅₀- 380.63±16.98 and 409.59±26.65 µg/ml) in scavenging free radicals. This property could possibly be related to its higher polyphenols content. Whereas ascorbic acid used as a reference standard showed scavenging potential with an IC₅₀ value of 16.00±0.19 µg/ml as shown in Table 2.

Lipid Peroxidation

Among tested four extracts of MD, MEMD & FFMD shows better dose dependent prevention

towards generation of lipid peroxides with IC₅₀ of 168.42±10.78 and 203.12±9.28 µg/ml respectively as compared AEMD & NFFMD (IC₅₀- 329.58±12.8 and 426.82±27.54). Ascorbic acid used as a reference standard showed scavenging potential with an IC₅₀ value of 21.64±1.70 µg/ml as shown in table 2.

Alkaline DMSO Scavenging Activity

MEMD & FFMD showed better activity with IC₅₀ value of 268.01±12.76 and 314.18±16.75 µg/ml respectively as compared to AQMD & NFFMD (IC₅₀- 317.87±20.7 and 386.98 ±22.01 µg/ml). The probable mechanism of scavenging the super oxide anions may be due to the inhibitory effect of MEMD & FFMD towards generation of superoxides in the *in vitro* reaction mixture (Table2).

Reducing Power

MEMD & FFMD showed moderate reducing power activity with IC₅₀ values of 332±16.76 and 345.78±22.60 µg/ml respectively, while other extracts was very less effective in reducing power activity as shown in table 2.

Hypolipidemic & Anti-Obesity Activity of MDR Extracts

The rats treated alone with atherogenic diet have shown hyperlipidemia and obesity significantly when compared with normal group animals in all biochemical investigation employed. Higher doses of the MEMD & FFMD (500 mg/kg) showed better results when compared to lower doses (250 mg/kg). The results found that MEMD (500 mg/kg) decreased serum TC, TG, LDL-C, VLDL-C, AST, ALT by 45.32%, 49.07%, 34.12%, 47.08%, 37.54%, 44.98%, 41.32% respectively, and increased HDL-C level by 75.04%, on the other hand FFMD (500 mg/kg)

decreased TC, TG, LDL-C, VLDL-C, AST, ALT by 37.87%, 44.20%, 24.41%, 42.33%, 33.46%, 32.65%, 22.81% respectively, and increased HDL-C level by 64.29% when compared to control group (figure 1 & 2).

Anti-Obesity Activity of MDR Extracts

The atherogenic diet treated animals have developed obesity when tested by various parameters. The development of obesity was found to be significant when compared with normal animals in all respect. Treatment with MEMD & FFMD resulted in decrease in weight of liver, spleen and heart at higher doses (500 mg/kg) as compared to organ weight in control group rats. However observed values were not statistically significant. The kidney weight, however, was not significantly different among the groups. MEMD (500 mg/kg) showed decrease in body weight by 95.07% whereas FFMD (500 mg/kg) has been decreased body weight by 85.92% when compared to control group as shown in table 3-5.

DISCUSSION

In the present study we have undertaken the evaluation of MDR fruit extracts for its potential hypolipidemic and anti-obesity activity. A phytochemical is a natural bioactive compound found in plant which is known in protecting many diseases. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the MEMD as well as in FFMD as shown in result section. Such compounds were known to possess potent antioxidant activity. These compounds are known to be biologically active and therefore could be responsible for their therapeutic effect. Although a high intake of polyphenols and flavonoids may significantly reduced the risk of hyperlipidemia. MEMD and FFMD showed better *in vitro* antioxidant & free radical scavenging activity among all the fractions as shown in table 2. However, none of the extracts were found to be more potent than the standard (ascorbic acid) since their IC₅₀ values were found to be higher. Due to its natural origin and potent antioxidant ability MEMD & FFMD could be used as a potential preventive

intervention for free radical-mediated diseases. In the present study, chronic atherogenic diet has been chosen for inducing hyperlipidemia and obesity. The possible mechanism involved in the atherogenesis in rat may be due to enhance cholesterol biosynthesis by increasing activity of HMGCoA reductase. In addition, this could be associated with a down regulation in LDL receptors by the cholesterol and saturated fatty acids in the diet.³⁰ The biochemical estimations shown that the extracts MEMD & FFMD increased the protective HDL-C level and decreased the atherogenic LDL and VLDL levels. The possible mechanism of test drug may involve increase of HDL-C, which can lead to the mobilization of cholesterol from peripheral cells to the liver.³¹

Treatment of AD fed rats with MEMD & FFMD (250/500 mg/kg) and reference standard atorvastatin (HMG CoA reductase inhibitor) showed a significant decrease of all lipid parameters and liver enzyme level and increase of serum HDL-C levels as shown in figure 1 & 2. The persons having chronic diseases are more likely to get obesity. In the present study, the anti-obesity effect of MEMD & FFMD were studied using the AD animal models of obesity as they have been reported to bear close resemblance to human obesity.²⁸ Therapeutic agents with both antihyperlipidemic and anti-obesity effects are therefore particularly beneficial. Results shows that AD group rats treated with MEMD & FFMD showed a time-dependent reduction in body weight, organ weight and increase in locomotors activity.

CONCLUSION

From the overall result of the biochemical and behavioural results, it could be inferred that MDR extracts showed hypolipidemic and antiobesity activity and activity was dose dependent and more at highest dose tested. Present studies reveal that MEMD and FFMD can be used as effective hypolipidemic and anti-obesity agent. The antioxidant property of extracts of MDR may be one of the reasons for the *in vivo* hypolipidemic activity produced by it. Further experiments are required to prove the mechanism and advantage

of MDR extracts over other drugs. Also the plants could be extended for the isolation and structure

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Table 1: Schedule of treatment for *in-vivo* activity on rats

Group	Treatment	Induction of hyperlipidemia and obesity
Normal	0.5% Sodium CMC (Vehicle) p.o for 25 days	Normal diet
Control	Vehicle p.o for 25 days	Atherogenic diet for 25 days
Atorvastatin	1.14 mg/kg, p.o. as suspension for 25 days	Atherogenic diet for 25 days
MEMD 250 mg/kg	250 mg/kg p.o. as suspension for 25 days	Atherogenic diet for 25 days
MEMD 500 mg/kg	500 mg/kg p.o. as suspension for 25 days	Atherogenic diet for 25 days
FFMD 250 mg/kg	250 mg/kg p.o. as suspension for 25 days	Atherogenic diet for 25 days
FFMD 500 mg/kg	500 mg/kg p.o. as suspension for 25 days	Atherogenic diet for 25 days

Table 2: Anti-oxidant activity of extracts of MDR fruits (IC₅₀ values in µg/ml)

Activity	IC ₅₀ values (µg/ml concentration)			
	MEMD	AEMD	FFMD	NFFMD
DPPH method	249.82±17.87	613.62±22.87	335.52±17.65	389.9±18.60
Hydrogen peroxide	293.80±12.87	380.63±16.98	367.78±22.87	409.00±26.65
Lipid peroxidation	168.42±10.78	329.58±12.80	203.12±09.28	426.82±27.54
Alkaline DMSO	268.01±12.76	317.87±20.77	314.18±16.75	386.98±22.01
Reducing power	332.00±16.76	463.57±21.77	345.78±22.60	542.76±25.00

Values are Mean±SEM, n=3

Table 3: Effect of extracts of MD on (body weight) AD induced obesity in rats

	Body weight (g)		
	Day 1	Day 26	% increase
Normal	171.87±8.98	190.75±10.76	9.89±1.65
Control	180.76±9.65	235.88±14.87 ^a	23.37±2.34 ^a
Atorvastatin	188.56±9.05	193.33±11.08 ^b	2.46± 0.77 ^a
MEMD 250 mg/kg	190.00±10.54	198.21±10.30 ^{b,c}	4.14±1.98 ^{b,c}
MEMD 500 mg/kg	176.66±6.77	178.73±9.76 ^{b,c}	1.15±0.67 ^{b,c}
FFMD 250 mg/kg	180.38±9.72	191.65±8.32 ^{b,c}	5.87±1.87 ^{b,c}
FFMD 500 mg/kg	178.90±8.50	185.05±12.54 ^{b,c}	3.29±1.21 ^{b,c}

Values represents Mean ± SEM (n = 6)

^a*P*<0.05, significant as compared to Normal

^b*P*<0.05, significant as compared to AD control group

^c*P*<0.05, significant as compared to Standard group

Table 4: Effect of extracts of MD on locomotors activities in AD induced obesity in rats

	Frequency of open field test*		
	Ambulation	Rearing	Grooming
Normal	69.0 ± 8.45	31.0± 3.1	6.0 ± 1.0
Control	32.0± 6.43 ^a	18.0± 2.1 ^a	2.0±1.8 ^a
Atorvastatin	60.0 ± 5.31 ^b	28.0 ± 2.6 ^b	6.0±1.3 ^b
MEMD 250 mg/kg	42.0± 7.23 ^b	20.0± 3.2 ^b	4.0±2.09 ^b
MEMD 500mg/kg	53.0± 3.45 ^{b,c}	25.0± 2.5 ^{b,c}	6.0± 0.8 ^{b, c}
FFMD 250 mg/kg	45.0± 4.2 ^b	20.0± 2.0 ^b	5.0± 2.2 ^{b, c}
FFMD 500 mg/kg	52.0± 6.7 ^{b,c}	24.0± 1.78 ^{b, c}	5.0± 1.2 ^{b, c}

*Tested for 5 minutes duration

Values represents Mean ± SEM (n = 6)

^a*P*<0.05, significant as compared to Normal group

^b*P*<0.05, significant as compared to the AD control group

^c*P*<0.05, significant as compared to the Standard group

Table 5: Effect of extracts of MD on (different organ weight) AD induced obesity

	Different organ weights (g)				
	Heart	Liver	Spleen	Kidney	
				Right	Left
Normal	0.57±0.21	5.28±1.12	0.71±0.12	0.6±0.09	0.58±0.07
Control	0.69 ±0.02 ^a	6.13±2.45 ^a	0.87±0.0 ^a	0.66±0.7 ^a	0.56±0.5
Atorvastatin	0.56±0.12 ^b	4.32±3.20 ^b	0.63±0.06 ^b	0.57±0.45	0.52±0.72
MEMD 250 mg/kg	0.65±0.2	5.59±1.70	0.78±0.12	0.62±1.02	0.53±0.86
MEMD 500 mg/kg	0.59±0.31 ^b	5.02± 1.45 ^b	0.65±0.09 ^b	0.61±1.04	0.48±0.04
FFMD 250 mg/kg	0.64 ±0.05	5.9 ±1.09	0.73±0.23	0.65±0.08	0.50±0.41
FFMD 500 mg/kg	0.60 ±0.20	5.1±2.00 ^b	0.63±0.51 ^b	0.63±1.02	0.51±0.09

Values represents Mean ± SEM (n = 6);

^a*P*<0.05, significant as compared to Normal;

^b*P*<0.05, significant as compared to AD control group; ^c*P*<0.05, significant as compared to Standard group

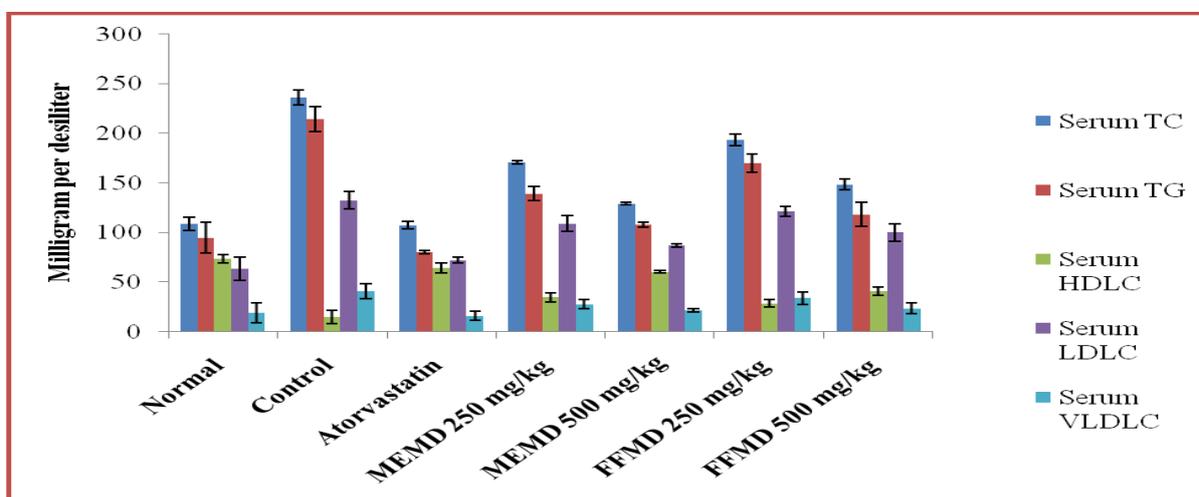


Figure 1: Hypolipidemic effects of extracts of MDR on atherogenic diet induced hyperlipidemic rats

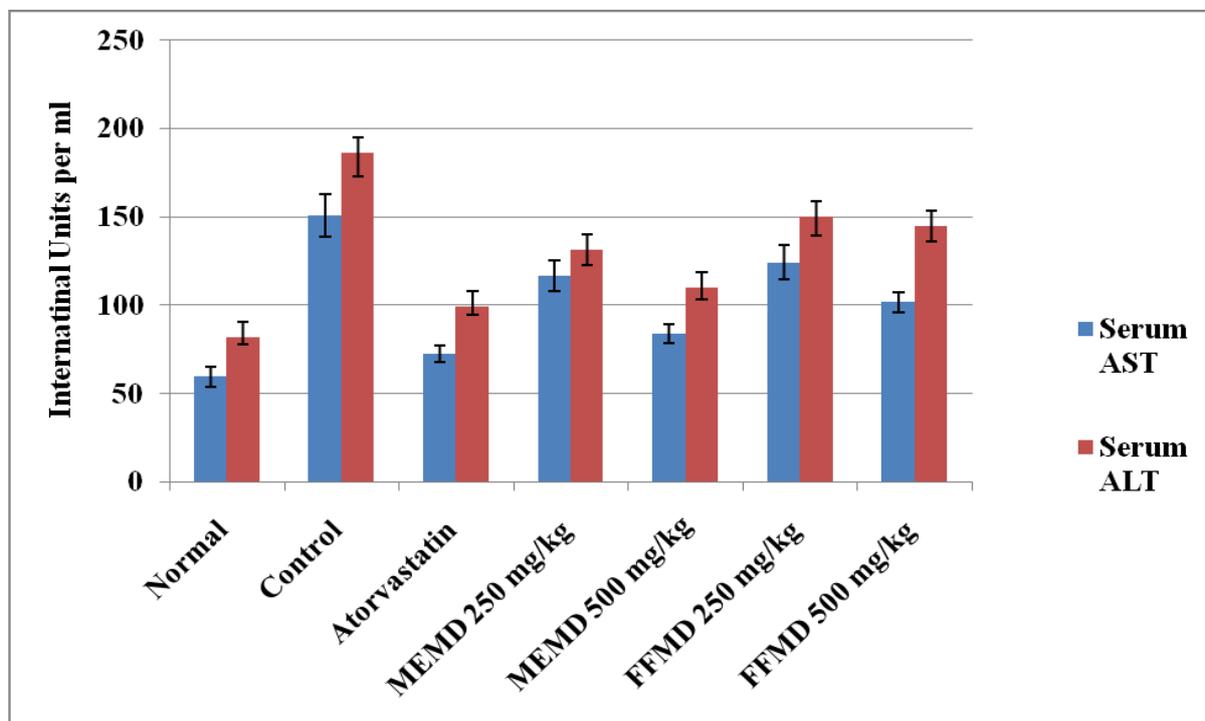


Figure 2: Hypolipidemic effects of extracts of MDR on atherogenic diet induced hyperlipidemic rats.

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