Original article:

Hypolipidaemic effect of rice bran oil and olive oil in hypercholesterolemic rats.

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ABSTRACT

Introduction: Hyperlipidaemia is a multifactorial metabolic disorder responsible for the development of cardiovascular diseases. Studies have demonstrated beneficial effect of rice bran oil (RBO) and olive oil in lowering LDL, VLDL and total cholesterol. The study was aimed at evaluating the hypolipidaemic effects of RBO and olive oil and comparing that with a standard hypolipidaemic drug, atorvastatin.

Materials and Methods: 30 healthy adult wistar rats of either sex were divided into 5(five) groups consisting of 6 animals each. Atherogenic diet was administered to all animals (except group A) for a period of 8 weeks along with atorvastatin (group C), RBO (group D) and olive oil (group E). Group B was the disease control and received atherogenic diet only. Serum lipid profile and histopathological examination of the abdominal aorta were performed.

Results: RBO and olive oil demonstrated comparable efficacy in reduction of LDL, VLDL and total cholesterol. The elevations in HDL levels were also similar. The serum levels of LDL, VLDL, HDL and total cholesterol in RBO and olive oil treated groups had statistically significant difference ($p<0.05$) than the atorvastatin treated group.

Conclusion: Rice bran oil and olive oil have prominent hypolipidaemic action, but it is not comparable to that of atorvastatin.

Keywords: Lipidaemia, Lipid, Heart, Metabolic disease.

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death worldwide. Hyperlipidemia is one of the main causes of atherosclerosis and atherosclerosis-induced conditions, such as CHD, ischemic cerebrovascular disease, and peripheral vascular disease. Coronary artery disease (CAD) is predicted to be the most common cause of death globally, including India, by 2020 [1]. Atherogenic dyslipidemia is referred to the triad of hypertriglyceridemia, low HDL (high density lipoprotein) cholesterol levels and elevated levels of small dense LDL (low density lipoprotein) cholesterol particles, which is now an increasing trend and is intimately associated with ischemic heart

disease, diabetes and hypertension which are together responsible for morbidity and mortality in humans to a large extent [2]. The allopathic hypolipidemic drugs, although available at large in the market, their popularity has been marred by numerous side effects, contraindications and exuberant cost. This has necessitated the search for alternatives [3]. Furthermore published reports have ascertained the isolation of certain nutrients that can be used as dietary supplements to meet up with the dietary imbalances in order to reduce the cardiovascular disease risk associated with hyperlipidemia [4] . There are various studies on the utilization of lipid lowering drugs in India. It has been reported that the utilization of lipid lowering drugs have significantly increased in last 10 yrs, but these drugs are expensive and their utilization is far below the expectation of many patients [5]. As diet enriched with saturated fatty acids and cholesterol contribute to the elevated lipid level of our population and many other developed countries around the world, dietary modification with reducing saturated fat intake and increasing unsaturated fat intake may prove beneficial. There are extensive literature related to atherosclerosis which have emphasized the beneficial effect of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) and deleterious influence of saturated fats. Various studies confirm the antiatherogenic potential of vegetable oils which contains PUFA and MUFA. Human studies showed that dietary PUFA reduces serum levels of all lipoproteins, whereas MUFA exerted their major effect only on the LDL fraction [6].

Vegetable oils containing unsaturated fatty acids which are widely used in some countries are rice bran oil (RBO), olive oil,

sesame oil, avocado oil, safflower oil etc. Rice bran oil (RBO) contains tocopherols, oryzanol and squalene. Oryzanol is mixture of ferulic acid esters of triterpene alcohols and 24 methylene cycloartanol which have been shown to lower the cholesterol absorption and aortic fatty streak formation [7]. Epidemiological evidences shows that Mediterranean diet containing rice bran oil is associated with lower incidence of CHD. Olive oil contains oleic acid and series of polyphenols which have antiaggregatory effect on platelets [8] and stimulate uptake of free radicals by leucocytes [9].

The present study is designed to evaluate the lipid lowering effect of two commonly used vegetable oils in wister strain rats fed with atherogenic diet. The two products are: Rice bran oil (RBO) and Olive oil. The significance of their hypolipidemic effect is compared with Atorvastatin.

AIMS AND OBJECTIVES

a) To study the hypolipidemic effect of rice bran oil in albino rats.

b) To study the hypolipidemic effect of olive oil in albino rats.

c) To compare the hypolipidemic effect of rice bran oil and olive oil with standard drug atorvastatin in albino rats.

MATERIALS AND METHODS

Ethical Approval:

The experiment was performed after due approval from Institutional Animal Ethics Committee (IAEC), Jorhat Medical College and Hospital.(Approval No. IAEC/JMCH/01/2015/002)

Drugs and Chemicals:

i. Cholesterol extra pure ($C_{27}H_{48}O$), procured from Research Lab. (Mumbai).

ii. Rice bran oil and Olive oil, procured from local health store.

iii. Atorvastatin procured from Torrent pharmaceuticals Ltd.

Experimental design:

The experiment was carried out for a period of 8 weeks. 30 healthy adult albino rats of wistar strain of either sex with an average body weight of 150-200gm were collected from the Central Animal House of Jorhat Medical College. Before the start of the experiment, the animals were allowed to acclimatize to the laboratory environment for one week and were housed individually in polypropylene cages under controlled environment at 25°C and 12 hours light and dark cycle. Animals were given free access to food and water ad-libitum, as per recommendation of CPCSEA (Committee for the purpose of control and supervision of experiment on animals).

The animals were divided into 5(five) groups consisting of 6(six) animals each.

Group A: Fed with standard diet

Group B: Fed with atherogenic diet (AD)

Group C: Fed with atherogenic diet (AD) + Atorvastatin

Group D: Fed with atherogenic diet (AD) + Rice bran oil

Group E: Fed with atherogenic diet (AD) + Olive oil.

Preparation of Atherogenic diet (AD) and test drugs:

i. AD was prepared by adding cholesterol powder (400mg/kg body weight/day) dissolved in 5ml of groundnut oil to standard diet [10].

ii. Atorvastatin dose calculation:

Adult human dose: 20mg/day in 70kg adult.

Now, by extrapolation method [11], the surface area ratio of 70kg man to 200gm rat comes to 0.018. Therefore, the total per day dose in a 200gm rat would be 20×0.018 = 0.36 i.e. 1.8mg/kg/day.

iii. Rice bran oil: 10% rice bran oil was prepared by adding 10gm of rice bran oil to 90gm of standard diet[12].

iv. Olive oil: 10% olive oil was prepared by adding 10gm of olive oil to 90gm of standard diet [13].

The test drugs were administered daily orally by feeding tube in the morning.

Estimation of serum lipid profile:

Under all aseptic conditions, blood samples were collected after overnight fasting at 0 week and at 8th week. During collection of blood, care was taken to prevent haemolysis. 2ml blood was taken from each animal via tail vein and collected in separate sterile empty vial (SEV) [14]. SEVs were centrifuged for 5 minutes at 3000rpm. The serum thus obtained was used for biochemical estimation. All the biochemical estimations were done using photo colorimeter.

Histopathological examination [15]:

Abdominal aorta from a representative rat in each group were dissected out and preserved in 10% formalin. After proper treatment with alcohol, these were embedded in paraffin. Small sections of the paraffin blocks were cut and stained with hematoxylin and eosin. The slides were observed under light microscope for presence of fat droplets.

RESULTS AND OBSERVATIONS

Serum lipid profiles were estimated at 0 week and 8th week. The results obtained in the study are summarized in tables 1, 2 and graphs 1, 2, 3, 4, 5, 1.1, 2.1, 3.1, 4.1 and 5.1. All the parameters are expressed as mg/dl. Total cholesterol, triglycerides and HDL levels were estimated using photo

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colorimeter. VLDL was calculated by dividing triglyceride by 5. LDL level was calculated by subtracting (HDL and VLDL) from total cholesterol. The results are presented as mean ± standard error of mean (SEM) of six animals in each group. One way analysis of variance (ANOVA) with Bonferroni's multiple comparison was used for determining the significance of intergroup differences. $p < 0.05$ was considered to be significant.

Serum Lipid profile at 0 weeks: (refer Table 1)

The serum TC, TG, HDL, VLDL, LDL values at 0 weeks in all the groups were found to bear no significant difference (p value > 0.05).

TABLE 1: Serum Lipid profiles of Groups at 0 weeks

	LIPID PROFILE (mg/dl) at 0 Weeks					
	Serum Total Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum High Density Lipoproteins (mg/dl)	Serum very Low Density Lipoproteins (mg/dl)	Serum Low Density Lipoproteins (mg/dl)	Atherogenic index of plasma (AIP)
GROUP A	65±2.683	78.17±2.272	23.33±1.116	15.63±0.4544	24.03±2.252	0.1634
GROUP B	63±1.932	77.83±3.545	21.33±0.9888	15.57±0.2894	23.10±1.770	0.2003
GROUP C	63±3.331	74.83±2.088	23±1.414	14.97±0.4177	25.03±0.8413	0.1501
GROUP D	60±2.129	73.33±2.060	21.33±1.085	14.67±0.4120	29±1.589	0.1743
GROUP E	64.50±2.500	77.50±2.029	23±1.183	15.50±0.4058	26.33±2.185	0.1652

Serum Lipid profile at 8 weeks: (refer Table 2)

At the end of the study, rise in all the parameters of the serum lipid profile except HDL was seen in group B (fed with AD). This was significantly higher than that of group A. In the atorvastatin treated group C, the serum lipid profile values (except HDL) were significantly lower than group B. The HDL level in group C was significantly higher than group B. The parameters (except HDL) in group D (animals fed with AD + rice bran oil) and group E (animals fed with AD + olive oil) were also significantly lower than in group B (animals fed with AD). The HDL levels in group D and group E were significantly higher than in group B. However, the levels in group D and E had significant statistical difference when compared to group C. There was no statistically significant difference between groups D and E.

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TABLE 2: Serum Lipid profiles of the Groups at 8 weeks

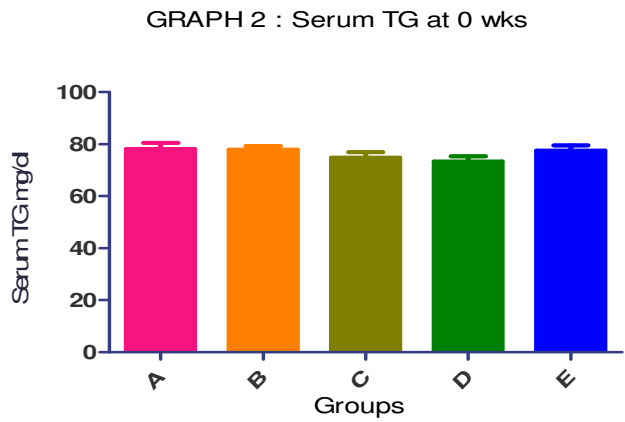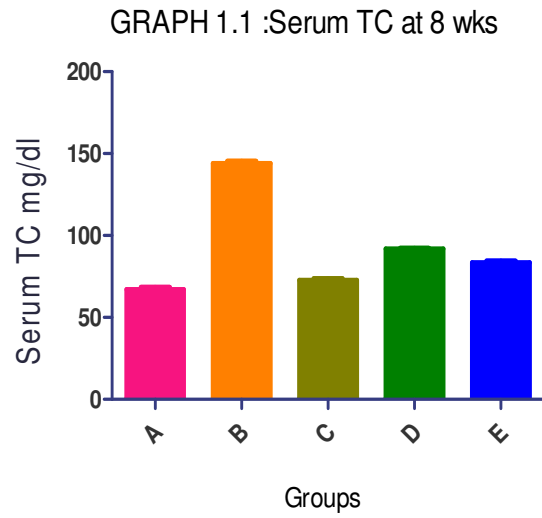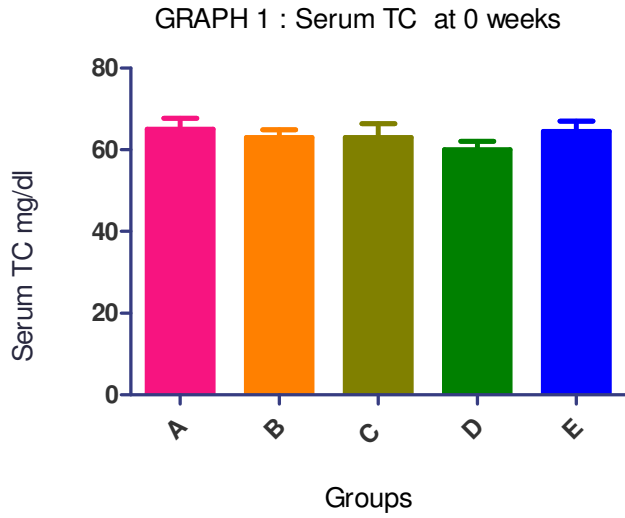| GROUP	LIPID PROFILE (mg/dl) at 8 Weeks					
	Serum Total Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum High Density Lipoproteins (HDL)(mg/dl)	Serum Very Low Density Lipoproteins (VLDL)(mg/dl)	Serum Low Density Lipoproteins (LDL)(mg/dl)	AIP (in ratio)
GROUP A(SD)	67 ± 1.390	81.33±0.5578	24.50±1.176	16.27±0.1116	26.23±0.6800	0.1589
GROUP B (AD)	144±1.438[a]	131.2±1.078[a]	15.50±0.6708[a]	26.23±0.2155[a]	102.3±1.620[a]	0.5654
GROUP C (AD+ATOR)	72.83±0.8597[b]	83.33±0.9888[b]	28.50±0.4282[b]	16.67±0.1978[b]	27.67±0.8846[b]	0.1038
GROUP D (AD+RBO)	91.83±0.4773[b,c]	92±0.5774[b,c]	23.17±0.6009[b,c]	18.40±0.1155[b,c]	50.17±0.7990[b,c]	0.2367
GROUP E (AD+OO)	83.33±1.116[b,c]	85.83±0.7032[b,c]	23.50±0.7638[b,c]	17.17±0.1406[b,c]	42.67±1.295[b,c]	0.2005
F	759	657.3	37.58	657.3	778	
df	4,25	4,25	4,25	4,25	4,25	
p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	

Values are expressed as MEAN ± SEM (n=6).

One Way ANOVA followed by Bonferroni's Multiple Comparison test is done.

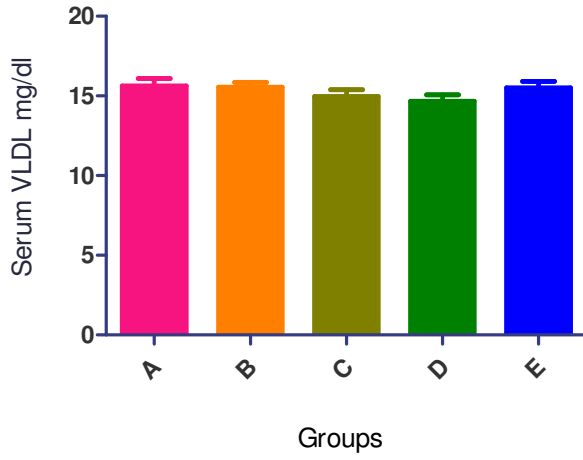[a]p <0.05   when compared to the Normal Control group i.e., Group A.

[b]p <0.05   when compared to the Experimental Control group i.e., Group B.

[c]p <0.05   when compared to the Standard drug group i.e., Group C.

GRAPH 1 : Serum TC at 0 weeks

GRAPH 1.1 :Serum TC at 8 wks

GRAPH 2 : Serum TG at 0 wks

GRAPH 2.1 : Serum TG at 8 wks

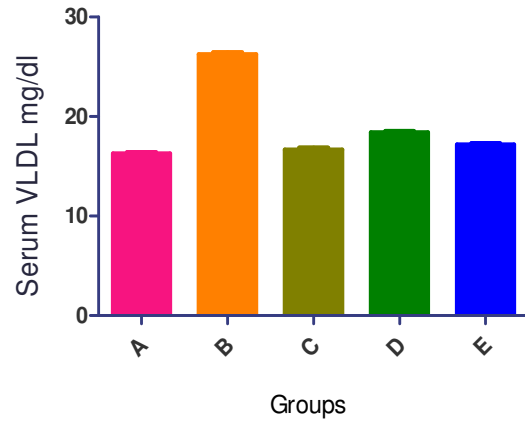GRAPH 3 :Serum HDL 0 weeks
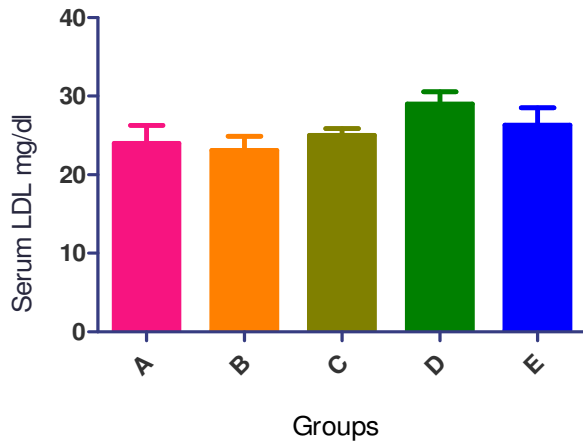
GRAPH 3.1 : SerumHDL 8wks
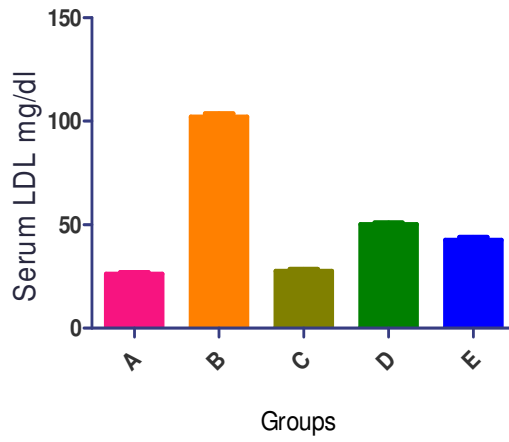
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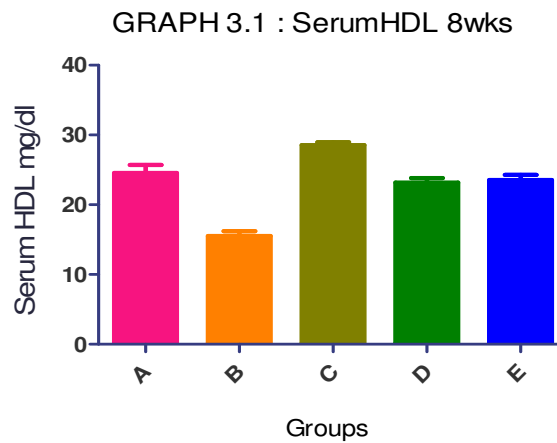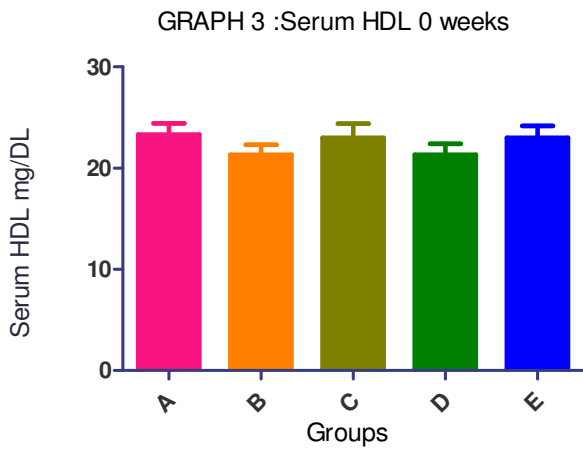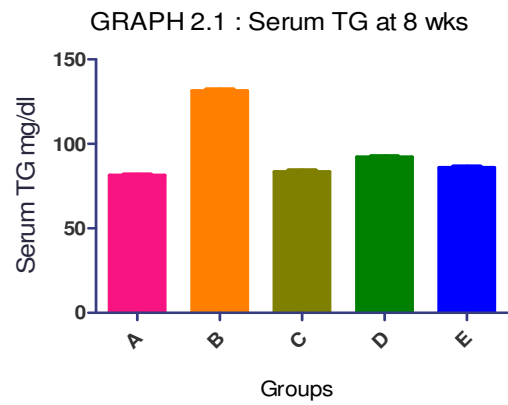
GRAPH 4 :Serum VLDL at 0 wks

GRAPH 4.1 : serum VLDL at 8 wks

GRAPH 5 : Serum LDL at 0 weeks

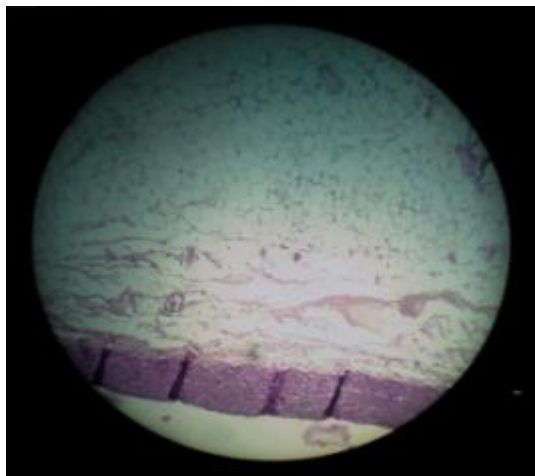GRAPH 5.1 : Serum LDL at 8 wks

Histopathological examination:
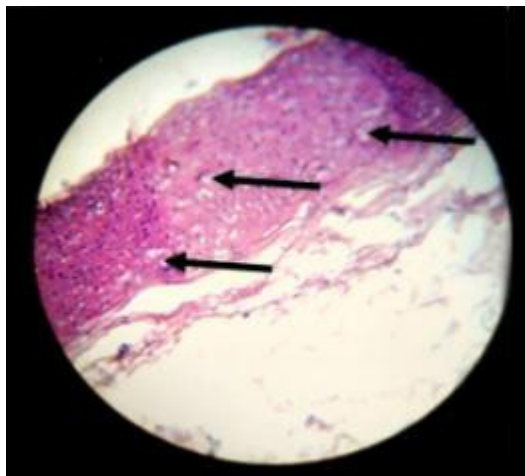

Fig.1. Normal tissue section (group A)

Fig.2. Section from the aorta exposed to AD showing thickening of the intimal layer with extensive lipid accumulation and collection of macrophages (group B)
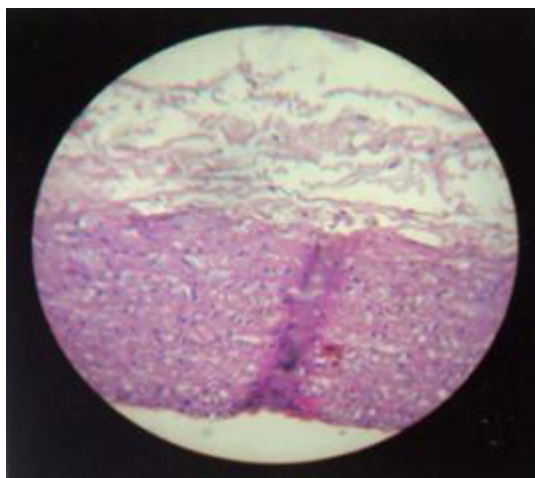

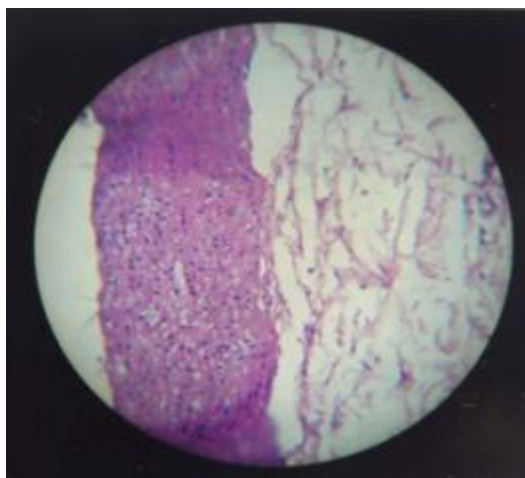**Fig. 3. Section from the aorta exposed to atorvastatin shows unremarkable tunica media (group C)**

Fig. 4. Section from the aorta exposed to rice bran oil shows mild lipid accumulation with minimal inflammatory cells (group D)
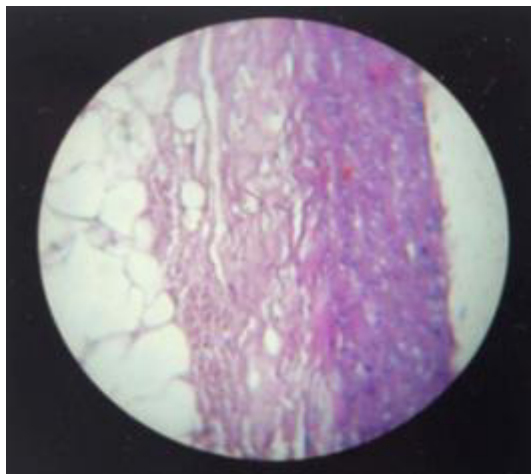
Fig. 5. Section from the aorta exposed to olive oil shows unremarkable tunica media with few inflammatory cells (group E)

DISCUSSION

Epidemiological studies carried out worldwide have recognized hyperlipidemia as a major risk factor in the development of various cardiovascular diseases. Vegetables oils like rice bran oil and olive oil containing unsaturated fatty acids and polyphenols are believed to lower lipid levels and prevent the development of coronary heart disease. In the present study, the hypolipidemic effects of rice bran oil and olive oil have been compared with the effects of atorvastatin in rats fed with atherogenic diet.

In this study, olive oil and rice bran oil demonstrated significant hypolipidemic activity. Their hypolipidemic effects were inferior to that of standard drug, atorvastatin. Rukmini C and Raghuram TC had demonstrated hypolipidemic effect of rice bran oil in cholesterol fed rats. They also reported significant rise in HDL levels following rice bran oil administration [7]. In the study conducted by Alhazza et al., olive oil was found to have significant hypolipidemic activity. It was hypothesized that the hypolipidemic effect of olive oil was due to its active constituent oleic acid [16]. Andreadou et al., also found significant hypolipidemic effect of olive oil owing to its constituent oleuropein [17]. Seetharamaiah GS and Chandrasekhara N found that rice bran oil lowers serum triglyceride and VLDL significantly in cholesterol fed animals [18]. In the histopathological examination of the aorta, extensive damage of the aorta evident from the thickening of the intimal layer and accumulation of lipids and inflammatory cells in the media was seen in animals fed with AD (group B). Marked improvement in the histopathological picture of the rats fed with AD and rice bran oil was seen. The tunica media was unremarkable except for focal lipid accumulation and minimal inflammatory cells. The improvement in the histopathological picture in the rats fed with olive oil and rice bran oil was almost comparable to that of rats fed with atorvastatin where there was almost intact endothelium with no or minimal presence of inflammatory cells. The laboratory findings of hypolipidemia in rats treated with rice bran oil and olive oil were substantiated by the findings of histopathology.

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CONCLUSION

Rice bran oil and olive oil have prominent and comparable lipid lowering effects on cholesterol, triglyceride, LDL and VLDL after 8 weeks of treatment in rats fed with atherogenic diet. They also significantly raise HDL. Administration of rice bran oil and olive oil prevented development of atherogenic changes in the aorta. However, their hypolipidaemic effect is inferior to atorvastatin.

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