Anti-Atherogenic and Hypocholesterolemic effects of Starch based Oleogels

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Abstract- Edible oleogels can be used for the delivery of lipid due to their composition, functional properties and structure. It is a novel way of replacing saturated fat in food which is otherwise harmful for health. Several types of edible oleogels have been characterized but only select systems have been used for oral delivery. There are certain properties of oleogel that renders it beneficial for delivery of the lipid. The objective of the study was to produce oleogels containing starch and lecithin as oleogelators and to assess its hypolipidemic effect on hypercholesterolemic rats. The uses of the oleogelation technique for the delivery of lipids have been used in oral delivery applications. The oil used in this case was a blend of rice bran and sesame oil in the ratio of 1:1 which has a known hypercholesterolemic and hypertensive property**.** Oleogel structure, mechanical strength, composition and gelator type are the important factors that affects the rate and extent of lipolysis of the oil. The oleogel produced was administered to both normal and hypercholesterolemic rats for 4 weeks. Results depicted that oleogel showed better hypolipidemic effect and anti-atherogenic effect in comparison to blended oil due to its improved delivery inside the body.

Key Words: oleogel, atherogenic index, hypercholesterolemia, hypolipidemia, rice bran oil and sesame oil blend

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1 INTRODUCTION

Degenerative diseases have been increasing among human beings as socio-economic environments and lifestyles have changed in the past years. People develop hypertension, obesity, hyperlipidemia which is all risk factors for cardiovascular diseases (CVD) in a cumulative way. CVD affects people of all ages, but is most frequent in middle age. Coronary heart disease is the most common type of CVD which accounts for over 30% of prevalent CVD and 47.7% of CVD death [1]. Coronary heart disease is caused by narrowing of coronary arteries which occur mainly due to hyperlipidemia. Hyperlipidemia is a condition where there is high concentration of cholesterol, triglyceride or low-density lipoprotein (LDL) in the blood. Hyperlipidemia also leads to hypertension i.e high blood pressure due to difficulty in movement of blood through blood vessels resulting from narrowing of blood vessel lumen. Scientific studies already suggest that using a blend of sesame oil and rice bran oil as cooking oil showed a significant antihypertensive and lipid-lowering action and had noteworthy additive effect with antihypertensive medication [2].

Presently lipid structuring by the formation of an oleogel has already established its physiological implications [3]. Structure, mechanical strength, composition and gelator type of the oleogel are factors that may affect the rate and extent of lipolysis of the material with which the oleogel is prepared. These in turn affect the delivery of lipid soluble molecules from the oleogel. They offer enhanced bio accessibility, prevention against bioactive recrytallisation and targeted or delayed bioactive release. Gelation offers the property to prevent precipitation of bioactives and slow lipolysis and release of nutraceuticals from crystalline and fibrillar oleogel networks.

The aim of this study was to structuring the blend of rice bran oil and sesame oil in the form of oleogels using sal starch and lecithin as gelators and tea polyphenol as antioxidant. The efficacy of the products was evaluated in affecting serum lipid levels (cholesterol, triglyceride, LDL and HDL levels) and cardiac lipid levels and atherogenic index in hypercholesterolemic subjects in comparison to blended oil itself.

2 MATERIALS AND METHODS

2.1 Sources

Rice bran oil and sesame oil were obtained from the local market.

2.2 Preparation of blended oil

A blend of rice bran oil and sesame oil was obtained by mixing both the oils in the ratio of 1:1 and final fatty acid composition was determined by GC [4].

2.3 Extraction of Sal Starch from Sal De-oiled Cake

Sal Deoiled Cake (Sal DOC) was steeped with four volumes of 1.25% sodium hydroxide (NaOH) solution at room temperature for 90 minutes. Thereafter the steeped solution was centrifuged at 5000 rpm for 15 minutes. The solid matter thus obtained consisting of mainly starch and fibre was washed further with water and centrifuged. The solid matter was steeped in water, wet ground and filtered after water washing (at least 4 washings) to obtain an aqueous suspension. This suspension was centrifuged and starch was obtained as residue. The crude starch thus obtained was washed successively with 0.1N HCl and 5% NaOH solution and water repeatedly. The starch obtained thereafter was bleached with sodium hypochlorite solution till there was a satisfactory decolourization. The bleached starch was washed with water till the chlorine odour was removed. Starch was finally dried at about 50-60ºC under vacuum over a tray drier [5].

2.4 Extraction of tea polyphenol by microwave assisted method

Polyphenol extraction from tea leaves was performed on microwave experimental setup using a closed vessel system; a total of 100 grams of tea according to the solvent-to-material ratio was put into a 500 mL vessel for extraction under different microwave assisted extraction (MAE) conditions. Samples were determined by Ultraviolet Visible (UV) spectrophotometer simultaneously [6].

2.5 Preparation of Oleogels

Oleogels with two organogelators (starch and lecithin with ratio of 1:1) were prepared at 10% w/w level. The decision of the selected levels was based on pre-experiments to determine the most suitable oleogels as spreadable products. Oleogels were prepared by mixing the blended oil with the gelators, starch and lecithin. Polyphenol was added (1% w/w) to the prepared oleogel to increase the anti-oxidative property and shelf life of the gel. Oleogel was

prepared with continuous stirring at 300rpm and cooled immediately at -20ºC and stored at 4ºC. After overnight setting at room temperature the oleogel was completely formed and then analyzed.

2.6 Gel characterisation

A. *Test for gelation:* The completely formed oleogels are taken in 10ml vials and pictures of the overturned vials are taken to observe the efficacy of gel formation [7].

B. *Hardness Evaluation:* Hardness or consistency of the samples was determined in terms of penetration yield value (g/cm^2) using a penetrometer with 40^o angle. The penetrometer is a fast and empirical method which is used in the determination of texture. The cone used for penetration was placed just above the sample before the cone was released. The time of penetration was 5s and depth was read directly from the instrument in 0.1mm unit. Yield values were calculated with the help of the equation given below:

   a. Yield value (g/cm^2)= KW/P$^{1.6}$

   b. Where K=constant (5840 for 40 cone angle); W=weight of the cone assembly (79.03); P=mean of penetration depth from three replicates (cm) [8]

C. *Microstructural Analysis:* Microstructural observation of lipid samples was conducted with a polarized light microscope which was attached to a digital camera. The oleogel samples (10µl) were placed on a microslide after melting the sample at 80ºC and then the samples were covered with cover slips. The microstructure analysis was performed of the samples which were stored at 4ºC [9].

2.7 Animals

Male Charles foster rats weighing 80-100 gms were grouped into six groups (six rats in each group) by random distribution and housed in individual cages, under a 12h light/dark cycle. Animals were given a fresh diet daily, and the leftover food was weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The animals had free access to food and water throughout the study. Each group of rats was fed for a total of 28 days. Table 1 shows the food composition of the different food groups.

Samples of the rat faecal matter were collected and analyzed 3 days prior to termination of the experiment. At the end of the experiment the feeding of rats was stopped and after 12h fasting, the rats were anesthetized by chloroform and 5ml of blood was taken from the heart. The plasma was obtained by centrifugation of the blood. The heart was removed, rinsed with ice-cold saline, blotted, weighed and stored at -20°C until analyzed. The experimental protocol was approved by the Animal Ethical Committee of Dept. of Physiology, University of Calcutta.

2.8 Analysis of Plasma Lipids

According to the standard methods, the lipid components such as total cholesterol, HDL cholesterol and phospholipid were analyzed using enzyme kits supplied by Merck India Ltd., Mumbai, India.

2.9 Analysis of Tissue Lipids

Cardiac lipid was extracted by the method of Folch et al [10]. One gram of tissue was homogenized with 1ml of 0.74% potassium chloride and 2ml of different proportions of chloroform and methanol for 2min and then centrifuged. The mixture was left overnight and the chloroform layer was filtered through a Whatman filter paper (no.1). The chloroform layer was dried, the tissue lipid contents were measured and the lipid was used for lipid analysis. The liver and brain lipids were used for the estimation of total cholesterol, HDL cholesterol, non-HDL cholesterol, TAG and phospholipid estimation by using standard kits.

2.10 Determination of Atherogenic Index

Atherogenic Index was calculated using the equation Total Cholesterol/HDL Cholesterol.

2.11 Bioassay of Total Cholesterol in Faeces

The weight of the faeces was recorded. The lipid was extracted from the faeces collected by the use of Soxhlet method. The lipid was then used for the determination of total cholesterol by using commercial kits.

2.12 Histological Study of Cardiac Tissue

Permanent preparations were made using routine methods. The cardiac tissues were fixed in 10% buffered formalin. The tissues were subsequently dehydrated in upgraded concentrations of alcohol, cleansed in xylene, impregnated and embedded in paraffin wax. Several sections of 3-6µ were cut using a microtome. The sections were stained with haematoxylin and eosin.

2.13 Statistical Analysis

All the data were expressed as mean ±SEM. Two ways ANNOVA was used to test the differences between control and experimental subjects.

3 RESULTS

3.1 Fatty acid composition of blended oil

The fatty acid composition of the blended oil is given in Table 2.

3.2 Oleogel Characterization

The oleogel prepared in this study has a smooth structure and good spreadability. The oleogel has a homogeneous texture and a considerable amount of stiffness and greasiness. Fig. 1a shows the pictures of the oleogel produced which exhibited the physicochemical properties of oleogel. Undesirable amount of greasiness, oiliness, grittiness, tackiness, stiffness or stickiness attributes uneasy feel and also limit the ability of the oleogels to be released inside the body. The study depicts that the oleogel used in this study has a good appearance and is very stable.

3.3 Microstructure characteristic of oleogel formulation

Aggregation processes of oleogel forming materials through self assembly and crystallization are the important properties of oleogel formation. Fig. 1b depicts the microstructures of the oleogel formed. From the figure it is evident that the oleogel showed formation of crystal aggregates i.e. network of fat crystals as shown by their microstructures where the crystal appears as dark spots. The figure showed the formation of small crystal aggregates in the starch oleogel network. The oleogel formed showed the presence of self assembly structures in their crystal networks. These aggregates are identified as sphere shaped structures called spherulites.

3.4 Effect of samples on hardness

The hardness of oleogels in general is a contributory factor of oleogel characteristics such as appearance, workability, spreadability and oil exudation. The hardness of oleogels usually increases due to the aggregation of fat crystal network. The yield value of the oleogel sample is 100.9±1.2 g/cm^2.

3.5 Effect of Dietary Lipids on Growth Parameters

The fat level was kept constant at 20% in all the dietary groups. The amount of diet consumed in the different groups was comparable. The effect of feeding dietary lipids on Body Weight Gain of normal and hypercholesterolemic rats is shown in Table 3 and 4. There was no significant difference in the food intake of the different groups both in the normal and hypercholesterolemic rats. However there was significant difference between the body weight gain of normal and hypercholesterolemic group ($p<0.01$). There was no significant difference between the body weight gain of the group fed with groundnut oil, blended oil and oleogel in the normal case. On the other hand the body weight gain decreased on feeding the rats with both blended oil and oleogel in hypercholesterolemic condition. The body weight gain was increased significantly by feeding the rats with 1% cholesterol in hypercholesterolemic group. The food efficiency ratios of the normal rats fed with blended oil and oleogels were higher compared to that of the rats fed with control oil. The food efficiency ratio of the control hypercholesterolemic rats was slightly lower than that of hypercholesterolemic rats fed with blended oil and oleogel.

The effect of dietary lipids on organ weights in normal and hypercholesterolemic cases is illustrated in Table 5. In normal case there was a significant decrease ($p<0.05$) in the weight of the cardiac tissue of the rats fed with blended oil and oleogel in comparison with the control diet. Similar results were also found in hypercholesterolemic condition. The decrease was more in case of rats fed with the oleogels.

3.6 Effect of the Different Dietary Lipids on Lipid Parameters in Plasma

The plasma lipid profile of normal rats fed with dietary lipids is shown in Table 6. The type of fat consumed both in the form of blended oil and oleogel altered the cholesterol concentration in plasma. Rats fed control groundnut oil had plasma total cholesterol of 91.39mg/dL which was significantly decreased to 86.72mg/dL and 76.27mg/dL by feeding them with blended oil and oleogel respectively. Thus the decrease in total cholesterol was much more in case of oleogel. The similar results were found in case of estimation of non-HDL cholesterol levels. However there was no significant change in the triacylglycerol levels. The level of HDL cholesterol, which was known as good cholesterol, increased significantly ($p<0.05$) by incorporating blended oil and oleogels in the diets of the rat. The increase in the HDL level was much more in case of oleogel in comparison with the blended oil.

The plasma lipid profile of the hypercholesterolemic rats fed with dietary lipids is also

shown in Table 6. The levels of all the lipid parameters increased in case of hyperlipidemia in comparison with normal case. Treating the rats with the experimental oils again decreased the levels of plasma total cholesterol and non-HDL cholesterol with not much change in triacylglycerol levels and increased the level of HDL cholesterol ($p<0.05$). Here also the hypolipidemic effect was much more in case of oleogel in comparison with blended oil ($p<0.01$).

3.7 Effect of the Different Dietary Lipids on Lipid Parameters of Heart

The heart is an important site for lipid deposition. The cardiac lipid profile of rats of different groups is shown in Table 7. Rats fed with control oil had total cholesterol of 24.58mg/g tissue which was significantly increased to 33.28mg/ g tissue by feeding them high cholesterol diet. Both the blended oil and oleogel brought about a decrease in total cholesterol which was much more in case of oleogel both in normal and hypercholesterolemic cases. The oleogels lowered the levels of triacylglycerol significantly ($p<0.05$). Phospholipid levels in the heart decreased in hypercholesterolemia, but administration of blended oil and oleogel increased its level. In this case also the effect of oleogel was far better.

3.8 Changes in Atherogenic Index

Fig 2 shows the change in atherogenic index of the normal and hypercholesterolemic rats fed with different dietary groups. It is evident from the figure that control oil depicts a higher rate of atherogenic changes in comparison to blended oil and oleogel. Oleogel however showed the least atherogenic changes both in normal and hypercholesterolemic rats.

3.9 Effect of Oleogel on the content of cholesterol in Faeces

To determine the effect of oleogel and blended oil on the excretion and conversion of cholesterol, faeces of rats were collected on the third day prior to termination of the study. As depicted in the Table 8, there was no significant change in the dry weight of the faeces of rats among all the groups. There was a change in the level of excretion of cholesterol of both normal and hypercholesterolemic rats. The group fed with blended oil and oleogel had greater excretion of cholesterol through faeces in comparison to the group fed with control oil. However the level of excretion was more in case of the group fed with oleogel than the group fed with blended oil.

3.10 Histopathological changes in the heart

Microscopic examination of the cardiac cells of the six groups was performed and the histopathological slides are shown in Fig 3. Fig 3a highlighted the cardiac histology of the control rats. Fig 3b highlighted the abnormal fatty change in the heart due to hypercholesterolemia. Fig 3c and d highlighted the histology of normal rats fed with blended oil and oleogel. Fig 3e and f highlighted the treatment of hypercholesterolemic rats fed with blended oil and oleogel. The fatty change was fully cured in case of the treatment with oleogel. The effect of treatment of the rats with oleogel was greater in comparison with blended oil.

4 DISCUSSION

In recent years, increasing attention has been paid to the development of new natural agents with lipid-lowering activity [11-13].

The biologically active fat soluble molecules like polyunsaturated fatty acids, phytosterol, carotenoids that are naturally present in foods are associated with positive health benefits [14]. Consumption of these biologically active compounds is associated with protection against cardiovascular disease [15-16]. Delivery of these biologically active molecules is therefore challenging and therefore designing of these moieties are required to enhance their bioavailability by overcoming limitations in matrix compatibility, solubility or chemical stability. This is because the biologically active molecules are lipophilic in nature. The bioaccessibility of a molecule is defined as the fraction released and available to be absorbed in the intestinal tract. Bioaccessibility is specifically a hurdle for fat soluble molecules which are insoluble in all aqueous environment of the digestive tract. Thus these fat molecules should be solubilised into mixed micelles in the aqueous phase to be able to be absorbed into epithelium cells of the small intestine. Because lipid digestibility is linked with the solubilisation and uptake of lipid soluble molecules the structural and compositional design of oleogels plays an important role in the application of the gels as delivery material for bioactive lipid molecule.

The objective of the present study was to investigate the role of blended oil as such and in the form of oleogel in preventing hypercholesterolemic atherosclerosis. The blended oil chosen in the study was a blend between rice bran oil and sesame oil. Literature review depicts that a blend of sesame oil and rice bran oil lowers blood pressure and improves the lipid profile in hypertensive patient. The oleogel is successfully produced as shown by the characterization study of the oleogel. The results show that oleogel was more effective in lowering plasma and cardiac lipid parameters than blended oil both in normal and

hypercholesterolemic cases. The reason for this observation was that when the blended oil was delivered in the form of oleogel it was protected from chemical degradation such as oxidation and thus the biological activity of the blended oil was retained. In the form of oleogel the oil is protected before they reach the site of absorption. Another possible reason was better bioaccessibility of blended oil when delivered in the form of oleogel. This was due to micellar solubilisation which is an important step for absorption of non-water soluble molecules like oil. The study also depicted that oleogels reduces the level of cholesterol in the blood through an increase in faecal cholesterol excretion.

The severity of the atheromatous lesions in heart as depicted by the histological investigation was associated with hypercholesterolemia. There were investigators who had similar results [17]. Hypercholesterolemic diet produced intimal wall thickening that contained foam cells similar to those observed by others [18]. Blended oil and oleogels reduced the extent of development of atherosclerotic index in hypercholesterolemic rats. Oleogels produced a better protective effect due to increased bioavailability of blended oil. This observation is in line with other several studies conducted by other investigators [19]. Therefore oleogels helps in reduction of atherosclerosis.

5 CONCLUSION

In conclusion it could be inferred that oil gelation techniques can improve the availability and absorption of the oil. The results suggested that hypercholesterolemic atherosclerosis is associated with an increase in lipid profile and decrease in cholesterol excretion and oleogels are effective in reducing hypercholesterolemic atherosclerosis by lowering serum levels of cholesterol and non-HDL-C and raising serum levels of HDL-C. The effect of oleogels in preventing hypercholesterolemic atherosclerosis and lowering coronary heart disease was relatively more in comparison with the blended oil fed as such. Thus the need for gelation of oil to provide a better matrix structure is important for the benefits of protection and controlled release of the oil moiety within the biological system.

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TABLE 1

FOOD COMPOSITION OF DIFFERENT DIETARY GROUPS

Sl.No.	**Dietary Group**	**Food Composition**
1	Normal control	Stock diet with 20% groundnut oil
2	Control fed with experiemental oil	Stock diet with 20% blended oil
3	Control fed with oleogel	Stock diet with oleogel prepared by using 20% blended oil
4	Hypercholesterolemic control	Stock diet with 20% groundnut oil and 1% cholesterol
5	Hypercholesterolemic fed with blended oil	Stock diet with 20% blended oil and 1% cholesterol
6	Hypercholesterolemic fed with oleogel	Stock diet with oleogel prepared by using 20% blended oil and 1% cholesterol

TABLE 2

FATTY ACID COMPOSITION OF BLENDED OIL

Fatty acid / ple	**Fatty Acid (% w/w)**			
	$C_{16:0}$	**$C_{18:0}$**	**$C_{18:1}$**	**$C_{18:2}$**
Blended Oil	19.48±0.11	4.12±0.09	43.65±0.19	32.75±0.22
Oryzanol content	0.66±0.02%			

Values are Mean±SEM

TABLE 3

EFFECT OF FEEDING DIETARY LIPIDS ON BODY WEIGHT GAIN OF NORMAL RATS

Parameters	**Control Oil**	**Blended Oil**	**Oleogel**
Food intake(g/day/rat)	9.94±0.87 [a]	9.44±0.07 [a]	9.64±0.12 [a]
Body weight gain(g)	34.17±0.99 [a]	30.09±0.45 [a]	27.66±0.67 [b]
Food efficiency ratio	0.29±0.01[a]	0.31±0.02 [a]	0.35±0.01[a]

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a row are statistically significant ($p<0.01$)

TABLE 4

EFFECT OF FEEDING DIETARY LIPIDS ON BODY WEIGHT GAIN OF HYPERCHOLESTEROLEMIC RATS

Parameters	**Control Oil**	**Blended Oil**	**Oleogel**
Food intake(g/day/rat)	9.05±1.32 [a]	9.27±0.98 [a]	8.91±0.25 [a]
Body weight gain(g)	42.51±0.46 [a]	30.14±0.99 [b]	27.83±1.21 [c]
Food efficiency ratio	0.21±0.01 [a]	0.30±0.01 [a]	0.32±0.03 [a]

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a row are statistically significant ($p<0.01$)

TABLE 5

EFFECT OF FEEDING DIETARY LIPIDS ON ORGAN WEIGHTS IN NORMAL AND

HYPERCHOLESTEROLEMIC RATS (G/100G BODY WT)

Organs	**Control Oil**		**Blended Oil**		**Oleogel**	
	N	**H**	**N**	**H**	**N**	**H**
Heart	3.64±0.02[a]	3.94±0.22[b]	3.04±0.11[c]	3.26±0.10[d]	2.91±0.02[c]	3.39±0.01[d]

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a row are statistically significant ($p<0.01$) N-normal case, H-hypercholesterolemic case

TABLE 6

PLASMA LIPID PROFILE (MG/DL) OF NORMAL AND HYPERCHOLESTEROLEMIC RATS FED WITH DIETARY LIPIDS

Parameters	**Control Oil**		**Blended Oil**		**Oleogel**	
	N	**H**	**N**	**H**	**N**	**H**
Total cholesterol	91.39±2.70[a]	154.15±2.98[b]	86.72±0.23[c]	109.93±1.89[d]	81.27±1.15[e]	99.06±1.70[f]
HDL-cholesterol	56.64±2.21[a]	28.86±2.11[b]	57.14±0.12[c]	46.05±1.85[d]	58.44±0.02[e]	49.07±0.99[f]
non-HDL cholesterol	15.03±1.23[a]	101.93±0.16[b]	10.19±1.56[c]	40.50±0.99[d]	3.73±0.98[e]	27.39±2.55[f]
Triacylglycerol	98.60±1.22[a]	116.79±1.29[b]	96.93±2.34 [a]	116.92±2.10[b]	95.51±3.76[a]	113.03±3.28[b]

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a row are statistically significant ($p<0.05$) N-normal case, H-hypercholesterolemic case

TABLE 7

CARDIAC LIPID PROFILE (MG/G TISSUE) OF NORMAL AND HYPERCHOLESTEROLEMIC RATS FED WITH DIETARY LIPIDS

Parameters	**Control Oil**		**Blended Oil**		**Oleogel**	
	N	**H**	**N**	**H**	**N**	**H**
Total cholesterol	24.58±0.95[a]	33.28±1.52[b]	21.22±0.99[c]	30.17±1.02[d]	17.29±1.50[e]	26.11±0.82[f]
triacylglycerol	95.28±0.21[a]	150.22±1.01[b]	92.25±0.20[c]	120.05±1.56[d]	90.22±0.55[e]	100.11±1.99[f]
phospholipid	108.85±1.22[a]	80.56±2.10[b]	118.96±1.88[c]	85.33±0.98[d]	100.56±2.01[e]	95.28±1.06[f]

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a row are statistically significant ($p<0.05$) N-normal case, H-hypercholesterolemic case

TABLE 8

AMOUNT OF CHOLESTEROL EXCRETED IN THE FAECES BY FEEDING THE RATS WITH DIFFERENT DIETARY GROUPS

Group	**Dry weight of faeces (g/day)**	**Cholesterol (mg/g)**
Normal	0.92±0.06	50.31±0.11
Hypercholesterolemic	0.87±0.03	22.71±0.29
Normal Blended Oil	0.90±0.02	55.82±0.21
Hypercholesterolemic Blended Oil	0.92±0.05	30.38±0.28
Normal Oleogel	0.91±0.04	60.82±0.41
Hypercholesterolemic Oleogel	0.88±0.02	37.90±0.08

Values are mean±S.E.M (n=6 rats) Values not sharing a common superscript within a column are statistically significant ($p<0.05$)

Fig 1 a

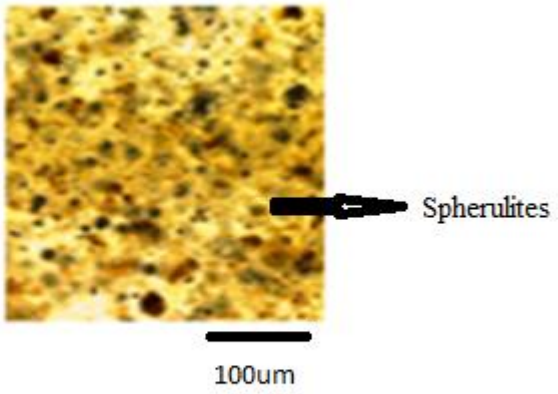

Fig 1 b

Fig. 1a, Pictographs of Oleogels formed by Olegelators (10%)

Fig. 1b, Pictographs showing microstructure of oleogels

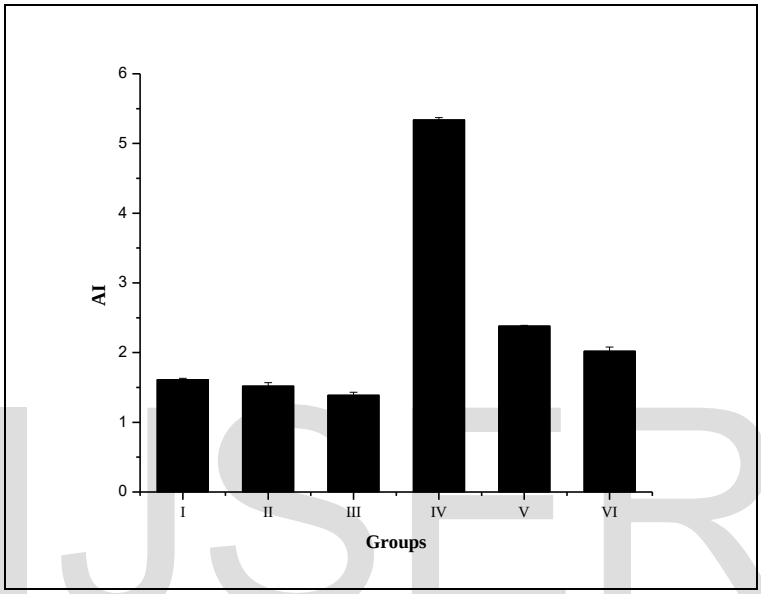

Fig. 2, Changes in Atherogenic Index [I: Control; II: Blended Oil; III: Oleogel; IV: Hypercholesterolemic Control; V: Hypercholesterolemia+Blended Oil; VI: Hypercholesterolemia+Oleogel]

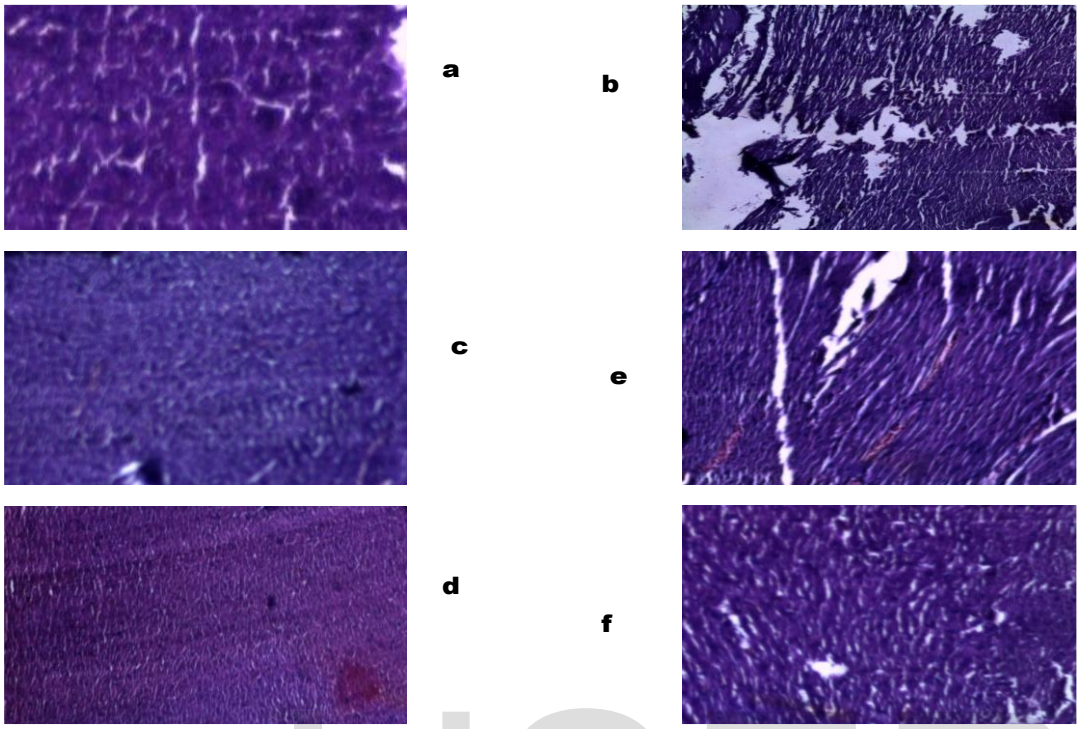

Fig. 3, Histopathological Change in Heart

a- Control; b- Hypercholesterolemic Control; c- Blended Oil; d- Oleogel; e- Hypercholesterolemia + Blended Oil; f- Hypercholesterolemia + Oleogel