EFFECT OF COCONUT OIL AND FISH OIL ON LIPID PROFILE AND ANTIOXIDANT STATUS IN RATS

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DECLARATION

I hereby declare that this thesis, entitled **"EFFECT OF COCONUT OIL AND FISH OIL ON LIPID PROFILE AND ANTIOXIDANT STATUS IN RATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"EFFECT OF COCONUT OIL AND FISH OIL ON LIPID PROFILE AND ANTIOXIDANT STATUS IN RATS"** is a record of research work done independently by **Sreeji K. P.,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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1. INTRODUCTION

Adequate amount of carbohydrates, proteins, lipids, vitamins and minerals are essential in the diet for a healthy living. Lipids include both fat and oil, while the former is a solid and latter a liquid at room temperature. Appropriate amount of fat to be included in diet is a topic of controversy. The Food and Drug Administration (FDA) recommends that 30% or less of calories consumed daily should be from fat, while others recommend that not more than 10% of a person's daily calories should come from fat.

Saturated fats comprise about 50% of cell membranes. Some proportion of saturated fats are found in all fats and oils, irrespective of their origin, they are probably the most maligned fats in the popular media today and are often blamed for "clogging arteries" leading to cardiac diseases.

Among the vegetable oils, coconut oil has a very high content (90%) of saturated fatty acids. Coconut kernel and oil are consumed mainly by the people of Philippines, Indonesia, India, Brazil, Thailand, Vietnam, SriLanka, Malaysia and other tropical places, for thousands of years. In India, southern states like Kerala, Tamil Nadu, Karnataka and Andhra Pradesh are well known for cultivation and production of coconut. Coconut oil was once prevalent in European countries and was a favourite in the baking industry. But a negative campaign against saturated fats in general led to most food manufacturers abandoning coconut oil in recent years in favor of hydrogenated polyunsaturated oils.

Coronary artery disease (CAD), also known as coronary heart disease (CHD) or coronary atherosclerosis is the world's leading killer, accounting for 16.7 million or 29.2 per cent of total global deaths in 2003. In 1990 there were an estimated 1.17 million deaths from CHD in India, and the number is expected to almost double to 2.03 million by 2010. Recently, an increased incidence of CHD was reported from Kerala. The last 30 years has seen a remarkable transition in

Kerala. Cardiovascular death is 50% of the total death and by 2020 it is predicted to go up to 2/3 of the total death (Cardiological Society of India, 2009). One of the main reasons for this is believed to be the consumption of coconut kernel and oil, which is a rich source of saturated fat.

Consumption of coconut kernel and oil have been the subject of controversy for nearly three decades. Very extreme positions have been taken on the consumption of coconut oil, due to the fear among people on the development of CHD. Various studies suggested an increased risk of CHD to elevated levels of serum cholesterol, which in turn is attributed to increased intake of saturated fats.

However, a fear complex has been created among the general public that consumption of coconut oil results in elevated cholesterol levels, which made the people of Kerala to shift from coconut oil to alternate cooking oils. This myth was primarily due to equating coconut oil with saturated fat without knowing that saturated fat in coconut oil are of the short and medium chain fatty acids, while fats that cause heart disease are saturated fats with long chain fatty acids.

Most commercial grade coconut oils are made from copra. Copra is basically the dried kernel (meat) of coconut. It can be made by: smoke drying, sun drying, or kiln drying. The standard end product made from copra is refined, bleached, and deodorized (RBD) coconut oil.

Coconut oil can also be prepared directly from coconut milk. The coconut milk is allowed to stand in a covered bucket for 24-36 hours and the crystal clear oil naturally separated from water retains the odour and taste. This oil is popularly known as Virgin coconut oil (VCO). Virgin coconut oil is growing in popularity as functional food oil and the public awareness of it is increasing.

People in the western coastal region of India usually use coconut oil for curried dishes. Coconut features in a lot of southern dishes, not just the coconut milk, but the oil and grated coconut are used as well. Usually coconut oil is seasoned during cooking with turmeric, onion, mustard and curry leaves.

Fish oil has high levels of omega-3 fatty acids and is believed that consuming fish oil (in any form) helps to regulate body cholesterol. Randomized clinical trials have shown that omega-3 fatty acid supplements can reduce cardiovascular events. Large-scale epidemiologic studies suggested that people at risk for CHD benefit from consuming omega-3 fatty acids from plants and marine sources. They can also slow the progression of atherosclerosis in coronary patients. The American Heart Association recommends the consumption of 1g of fish oil daily, preferably by eating fish, for patients with CHD. In fact, more studies are needed to confirm and further define the health benefits of omega-3 fatty acid supplements for preventing a first or subsequent cardiovascular event.

India has the third largest edible oil economy in the world. Vegetable oil consumption in the country is continuously rising and has sharply increased in the last couple of years to roughly 11.2 kg/head/year. This is still lower than the world average consumption level of 17.8 kg/head/year. The developed western world has a per capita consumption of 44 to 48 kg/year (Ramesh and Murughan, 2008).

Throughout the tropics coconut oil has provided the primary source of fat in the diets of millions of people for generations. It has various applications in food, medicine, and industry. Its heat stability makes it an excellent cooking and frying oil. But coconut oil had the misfortune of being labeled as a dietary troublemaker because of its high saturated fat content and many people avoid using it for that reason.

Though there are studies that indicate a positive correlation between consumption of coconut oil and development of CHD, most of the recent investigations conducted in animals as well as human beings contradict claims that coconut oil increases the risk of atherosclerosis and heart disease. Thus, there exists a controversy among the scientific community regarding the negative effects of coconut oil.

In this context, a study was designed in rats using various preparations of coconut oil viz., copra oil (RBD coconut oil), virgin coconut oil (VCO) and seasoned coconut oil (SCO) and fish oil (FO) with the following objectives:

1. To assess the alterations in serum lipid profile by the administration of commercial coconut oil, seasoned coconut oil, virgin coconut oil and fish oil.

2. To assess the antioxidant property of these oils in tissues of liver and heart.

This study will help to unveil the actual role of coconut oil on lipid metabolism thereby annihilate the misunderstanding on coconut oil consumption and its relation to atherogenesis.

2. REVIEW OF LITERATURE

2.1. CORONARY HEART DISEASE (CHD) AND OIL CONSUMPTION

A study conducted by Malhotra (1967) showed that mortality from CHD differed between employees of Indian railways across railway zones. Mortality rates were seen to be lowest in Northern zone (20/100000), higher in Eastern and central zones (50/100000 and 63/100000, respectively) and highest in Southern zone (135/100000).

Sarvotham and Berry (1968) carried out a prevalence survey of CHD in a town in the northern part of India, involving a house-to-house clinical and electrocardiographic examination of all the 2,030 persons above the age of 30 years residing in the area. They observed an increase in the prevalence of CHD with age, socio-economic status, sedentary nature of occupation and hypertension. They also noted that those with CHD were more obese than others, and the prevalence showed a positive correlation with sub scapular skin fold thickness in men.

Kinsella *et al*. (1990) reviewed the effects of dietary n-3 polyunsaturated fatty acid (PUFA) components of fish or marine oils, especially eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), on several phenomena associated with heart disease. The n-3 PUFA depressed plasma lipids, especially Triacylglycerols (TAGs), by inhibiting hepatic TAGs and possibly apoprotein synthesis. They also replaced arachidonic acid in phospholipid pools with EPA and DHAs. Thus PUFAs, when released, inhibit the enzymes, cyclooxygenase and lipoxygenase, thereby reduce the synthesis of eicosanoids by platelets and macrophages, particularly thromboxane A2 (TXA2) and leukotriene B4 (LTB4). Reduction of the proaggregatory, vasoconstrictive TXA2 decreases the thrombotic tendency of platelets.

Almendingen *et al.* (1995) compared the effects of partially hydrogenated fish oil (PHFO-diet), partially hydrogenated soybean oil (PHSO-diet), and butterfat (butter-diet) on serum lipids and lipoprotein - a (Lp (a)) in 31 young men who consumed the test diets for 19-21 days. All the test diets contained about 35% of energy from fat. They observed a significant elevation in the serum total cholesterol and LDL cholesterol and a significant decrease in HDL cholesterol on PHFO diet compared to PHSO diet. In comparison with butter fat diet the PHFO diet showed similar total and LDL cholesterol levels and significantly lower HDL cholesterol level. The ratio of LDL to HDL cholesterol was significantly higher on the PHFO-diet when compared to both other test diets and no significant difference was observed in triglyceride values. Level of Lp(a) was higher in PHFO and PHSO diets. It has been suggested that these effects were due to trans C18: l and the very long chain trans fatty acids in PHFO. These results indicated that consumption of PHFO increase the lipid risk factors responsible for CHD at least to the same extent as butterfat.

Gupta and Gupta (1996) reviewed studies determining CHD prevalence in India. They found that CHD prevalence increased in urban areas from 1% in the 1960s to 9% in the 1990s. Whereas in rural areas the rate increased from 2% in the 1970s to 4% in the 1990s.

Enas *et al.* (1997) reported that several factors have contributed to the acceleration of CHD epidemic in India, which includes demographic transition to an older population as a result of increasing life expectancy, confluence of both conventional risk factors and non-conventional risk factors.

A low saturated fat diet and maintenance of ideal body weight and waist circumference is advocated as an individual based preventive strategy for those with markedly elevated risk factors for CHD (Enas, 1998).

Hu *et al*. (1999) examined the association between intake of individual saturated fatty acids and their food sources and the risk of CHD in humans. They found that a higher dietary intake of saturated fatty acids, including 12:0, 14:0, 16:0, and 18:0, was associated with an increased risk of CHD, whereas intake of short to medium chain saturated fatty acids (4:0–10:0) was not. Higher consumption of red meat and high fat dairy products, the main sources of saturated fatty acids in the diet, was also associated with greater risk. In contrast, higher consumption of poultry and fish and low-fat dairy products was associated with a lower risk of CHD. They also found a strong inverse association between the ratio of polyunsaturated to saturated fat and the risk of CHD.

Gupta *et al.* (2000) in an observational study showed that serum total cholesterol ≥200 mg/dl was associated with increased long-term cardiovascular mortality in Indian CHD patients.

In a health study conducted in nurses, Hu *et al*. (2000) observed a substantial decline in the incidence of CHD in women from 1980 to 1994 and suggested that reduction in cigarette smoking, healthy diet, and postmenopausal hormone replacement therapy accounted for this. However, increase in obesity probably prevented a further decline in the incidence of coronary disease.

Frenoux *et al*. (2001) investigated the effects of a diet rich in PUFA (γ-Linolenic acid [GLA, 18:3(n-6)], EPA [20:5(n-3)] and DHA [22:6(n-3)] at the rate of 5.65, 6.39 and 4.94 g/kg dry diet, respectively) for 10 weeks on blood pressure, plasma lipid concentrations, total antioxidant status, lipid peroxidation and platelet function in spontaneously hypertensive rats. They observed a significant decrease in blood pressure, improvement in total antioxidant status and resistance to lipid peroxidation, reduction in platelet aggregation speed and lowered plasma lipid concentration. They suggested that PUFA supplementation enhanced protection against cardiovascular diseases.

Hu *et al*. (2001) reviewed on the relationship between dietary fat intake and risk of CHD in human subjects, with an emphasis on different major types of fat, n-3 fatty acids and the optimal balance between n-3 and n-6 fatty acids. They reported that the types of fat are more important than total amount of fat in determining the risk of CHD. Controlled clinical trials have also shown that replacing saturated fat with polyunsaturated fat is more effective in lowering serum cholesterol and reducing risk of CHD than simply reducing total fat consumption.

Aguilera *et al.* (2002) studied the effect of sunflower oil, virgin-olive oil and fish oil on progression of aortic lesions in rabbits maintained on a high cholesterol-saturated fat diet (3% lard and 1.3% cholesterol) for 50 days. Then for 30 days the diet was replaced with cholesterol free unsaturated fat diet containing either 1.75% sunflower oil, virgin-olive oil and fish oil. They observed that replacement of a high cholesterol-saturated fat diet by cholesterol free-unsaturated fat diet containing virgin olive, sunflower or fish oil did not regress atherosclerosis in rabbit. However, sunflower oil provoked a significant progression of the lesion development, whereas virgin olive oil and to a lesser extent fish oil did not cause any progression of the lesion.

Khot *et al*. (2003) reported that the four conventional risk factors of CHD such as, cigarette smoking, diabetes, hyperlipidaemia and hypertension, and the lifestyle behaviours have to be addressed and has great potential to decrease the worldwide epidemic of CHD.

Singh and Sen (2003) reported that the major risk factors for CHD are sedentary lifestyle, cigarette smoking, hypertension, high level of LDL cholesterol, low level of HDL cholesterol and diabetes mellitus. Other factors that influence are obesity, family history of premature CHD, insulin resistance, hyper-triglyceridaemia, small dense LDL particles, Lp (a), serum homocysteine and

abnormalities in several coagulation factors. Psychosocial and socioeconomic factors are also considered important.

Vanschoonbeek *et al.* (2003) reported that the antithrombotic potential of fish oil, rich in n-3 PUFA, in humans is attributed to a reduction in platelet activation, a lowering of plasma TAG and (vitamin K–dependent) coagulation factors and/or a decrease in vascular tone.

Ahmad and Bhopal (2005) reviewed that the incidence of myocardial infarction (MI) in urban India in the 14 years (1977-1991) remained as 6/1000 in males and 2/1000 in females. Prevalence range was higher in urban than rural areas in men (35–90/1000 v 17–45/1000) and women (28–93/ 1000 v 13–43/1000). No clear rise was observed in relation to age in men over a 27 year period (1977-2002), whereas a clear rise was reported in women.

Holmberg *et al*. (2009) investigated the effects of food choices on CHD over 12 years of follow up in rural Swedish men and identified a significant interaction between intake of fruits and vegetables and dairy fat. Daily intake of fruits and vegetables was found to be associated with a lower risk of CHD only when combined with a high intake of dairy fat. Low intake of dairy fat, choosing mostly whole meal bread or eating fish at least twice a week was not associated with a reduced risk of CHD.

2.2 COCONUT OIL

Gas chromatography (Helium as carrier gas at a flow rate of 5.4 ml/min) analysis of copra oil by Nevin and Rajamohan (2008) revealed its fatty acid composition as:

Fatty acids	Percentage
Caprylic acid (8:0)	8.15
Capric acid (10:0)	5.56
Lauric acid (12:0)	43.55
Myristic acid (14:0)	18.38
Palmitic acid (16:0)	8.25
Stearic acid (18:0)	2.65
Oleic acid (18:1)	6.70
Linoleic acid (18:2)	1.49
Arachidic acid (20:0)	0.086
Gadoleic acid (20:1)	0.042
Behenic acid (22:0)	0.018
Lignoceric acid (24:0)	0.065
Other fatty acids	5.06

The total polyphenol content of copra oil was estimated to be 64.4 mg/100 g oil.

2.2.1. Effect on consumption

Hostmark *et al.* (1980) compared the effect of diets containing 10% coconut oil and 10% sunflower oil on lipoprotein distribution in male Wistar rats. Rats fed on diet containing coconut oil showed significantly lower level of pre-beta lipoproteins (VLDL) and significantly higher level of alpha-lipoproteins (HDL) compared to the sunflower oil fed group.

Awad (1981) studied the effect of diets, containing 14% coconut oil, 14% safflower oil and 5% soybean oil (control) on accumulation of cholesterol in tissues in male Wistar rats, fed for a period of 4 weeks. The first two diets had 2% added corn oil with a total fat of 16%. Total tissue cholesterol accumulation for animals on the safflower oil diet was found to be six times greater than for animals on coconut oil diet, and twice that of the control animals.

Medium chain fatty acids (MCFAs) of coconut oil, which on reaching hepatocytes do not require carnitine acyltransferase for their transport across the inner mitochondrial membrane (Foster, 1984).

Studies conducted in Rhesus monkeys, on a diet containing 30% corn oil (polyunsaturated fatty acid) for three months when changed to a diet containing 30% coconut oil (saturated fatty acid) for nine months, showed that the level of plasma cholesterol rose maximally within one month of the changed diet and remained at that level over the ensuing 8 months. Plasma TAG and phospholipids were also elevated significantly during the period of saturated fat feeding. Concentration of all classes of plasma lipoproteins were increased without altering the lipoprotein size, suggesting an increase in particle number. Corn oil diet lowered HDL level, which might be associated with higher fractional catabolic rates of both apolipoprotein A-I (apo A-I) and apolipoprotein A-II (apo A-II) (Chong *et al*., 1987).

Carlson and Kottke (1991) demonstrated that short-term coconut oil (14%) feeding (within 3 days) greatly increased plasma apo A-I and HDL-cholesterol and significantly decreased the very low density lipoprotein (VLDL) cholesterol in Watanable (WHHL) rabbits. Elimination of coconut oil from the diet immediately decreased the apo A-I levels. No significant changes in plasma cholesterol or TAG levels were found during this period. A trilaurin supplemented diet also caused an immediate increase in apo A-I and HDL cholesterol levels (within 2 days) and returned to baseline levels shortly after the elimination of trilaurin from the diet (within 3 days). They suggested that lauric acid may be the active component in coconut oil that caused the increase in apo A-1 levels in rabbits as it is possible that free lauric acid may regulate the synthesis and secretion of apo A-I by mucosa cells which synthesize the majority of apo A-I in rabbits.

Heek and Zilversmit (1991) studied the mechanisms of hypertriglyceridaemia in rabbits fed for 27 days with 14% coconut oil/0.5% cholesterol (CNO/Chol) diet with those fed with 14% olive oil/0.5% cholesterol (OO/Chol) diet. They observed significantly high contents of lipid and protein in the VLDL and intermediate density lipoprotein (IDL) fractions of plasma from CNO/Chol diet fed rabbits compared with OO/Chol diet fed rabbits. However, the particle diameters of these lipoproteins in the two groups did not differ. They suggested that hypertriglyceridaemia and hypercholesterolemia in the CNO/Chol rabbits could primarily be due to an increased hepatic secretion of VLDL and a modest decrease in VLDL triacylglycerol clearance capacity.

Jones *et al.* (1994) reported that fatty acid or plant sterol levels of dietary oils consumed may play a role in the regulation of cholesterogenesis. Plant sterols or other compounds such as squalene found in plant oils may upregulate cholesterogenesis indirectly by depressing the absorption of cholesterol or directly by serving as a synthesis precursor.

Cox *et al*. (1995) studied the effects of coconut oil, butter and safflower oil on serum lipids and lipoproteins in moderately hypercholesterolaemic individuals who were provided with the experimental diets for 6 weeks containing the different test fats providing 50% of total dietary fat. Coconut oil and butter diets resulted in increased total and LDL cholesterol compared to the safflower oil diet. However, the levels of both the lipids were significantly lower in the individuals on coconut oil diet than on butter diet despite similar total saturated fatty acid composition and a higher content of cholesterol elevating fatty acids (lauric, myristic and palmitic acids) in coconut oil diet. It has been suggested that butter fat rich in palmitic acid, has a greater hypercholesterolemic effect than coconut oil, which is rich in lauric acid.

Schwab *et al.* (1995) investigated the effect of diets high in lauric acid (as coconut oil) and palmitic acids (as palm oil) for a period of 4 weeks on serum

lipids and lipoproteins and on glucose tolerance and insulin sensitivity in young healthy women. They observed that a substitution of 4% energy as lauric acid or palmitic acid for monounsaturated fatty acids had only non significant effects on the levels of serum lipids, lipoproteins and did not cause any alternations in glucose metabolism.

Beena *et al.* (1996) studied the biochemical response in rats to the addition of curry leaves (Murraya koenigii) and mustard seeds (Brassica juncea) to the diet. Supplementation of 10% curry leaves or 10% mustard seeds to a standard diet with 20% coconut oil for 90 days resulted in a reduction in total serum cholesterol, LDL, VLDL, an increase in the HDL, lower release of lipoprotein into the circulation and an increase in the LCAT activity.

Studies conducted by Suma (1998) in 258 human volunteers (age 18–65 years) showed that coconut oil consumption (15.40 g/head/day and average contribution of total fats were 16.3%/head/day) was beneficial in reducing LDL cholesterol by raising HDL cholesterol, when compared to groundnut oil.

Zulet *et al.* (1999) investigated the influence of a diet enriched with coconut oil (25% w/w) and cholesterol (1% w/w) on carbohydrate and lipid metabolism in rats for a period of 26 days. They observed significant increase in liver weight, liver cholesterol, total serum cholesterol and LDL, while serum HDL and TAG decreased significantly. It has been suggested that the hypercholesterolaemic diet might have caused inadequate synthesis of lipoproteins poor in TAG and rich in cholesterol.

Asai and Miyazawa (2001) investigated the effects of dietary supplemented curcuminoids on lipid metabolism in rats which were fed a high fat diet (15 g soybean oil/100 g diet) for 2 weeks. Supplementation of curcuminoids (1%) showed significant decrease in liver triacylglycerol and cholesterol concentrations and also lowered level for plasma triacylglycerols in the VLDL

fraction. Hepatic acyl-CoA oxidase activity was significantly higher in curcuminoid supplemented rats. They suggested that lipid lowering effect of curcuminoids might be due to alterations in fatty acid metabolism.

Hussain (2002) studied the effect of oral administration of aqueous extract of turmeric on blood glucose, lipid peroxidation and the antioxidant defense system in rat tissues like liver, lung, kidney and brain for a period of 8 weeks in streptozotocin induced diabetic rats. They observed a significant reduction in blood glucose, decreased free radical formation in the tissues and an increase in total haemoglobin content.

Pretreatment of rats with the ethanol extract of *Curcuma longa* (100 mg/kg) orally for 7 days prior to paracetamol dosing at 600 mg/kg statistically lowered the three serum liver enzyme activities (Alanine aminotransferase, Aspartate aminotransferase and Alkaline phosphatase). Moreover, treatment of rats with only the ethanolic extract of Curcuma longa (100 mg/kg) had no effect on the liver enzymes. The results suggested that ethanolic extract of Curcuma longa has potent hepatoprotective effect against paracetamol-induced liver damage in rats (Somchit *et al*. 2002).

Giudetti *et al*. (2003) assessed the differential effects of 15% coconut oil, abundant in medium-chain saturated fatty acids and 15% fish oil, rich in n-3 PUFAs, enriched diets fed for 3 weeks on the tricarboxylate carrier (TCC) in rat liver mitochondria. They observed that the TCC activity was markedly decreased in liver mitochondria of rats fed with fish oil diet compared to coconut oil fed group. This effect is thought to be due to reduced expression of the gene of the carrier protein.

Muller *et al*. (2003) reported that a diet high in saturated fatty acids (HSAFA) lowered postprandial tissue plasminogen activator antigen (t-PA) and thus potentially improved fibrinolysis compared with a highly unsaturated fatty

acid diet (HUFA) in humans in a 4 months experimental study. Thus a coconut oil based diet (HSAFA-diet) lowers postprandial t-PA antigen concentration, and this may favorably affect the fibrinolytic system and the Lp(a) concentration compared with the HUFA-diet.

Aoyama and Yamamoto (2007) studied the antioxidant activity and flavonoid content of Welsh onion (*Allium fistulosum*) and the effect of thermal treatment on them compared with those of yellow and red varieties onion (*Allium cepa*). The order of antioxidant activity in red onion followed by yellow onion, green Welsh onion and white Welsh onion. Total flavonoid content was highest in red onion. Major flavonoid of yellow and red onions was found to be quercetin, and that of green Welsh onion was kaempferol. Antioxidant activity of green Welsh onion was increased, but that of the other onions was found decreased during boiling for more than 15 minutes.

Arulselvan and Subramanian (2007) evaluated the possible protective effects of *Murraya koenigii* leaves extract against β-cell damage and antioxidant defense systems of plasma and pancreas in streptozotocin induced diabetes in rats. The levels of TBARS, enzymatic and non-enzymatic antioxidants were increased in diabetic rats which were brought back to near control levels after treatment with the extract.

Kesari *et al.* (2007) examined the effect of 1 month oral administration of *Murraya koenigii* aqueous leaves extract in normal and streptozotocin induced severe diabetic rats, at the dose of 300 mg/kg b.w., on various biochemical parameters. They found that in case of diabetic animals fasting blood glucose levels of treated animals reduced by 48.2% after 30 days treatment. A fall of 19.2 and 30.8% in total cholesterol and 22.97 and 37.1% in TAG levels were also observed in the case of treated normal as well as diabetic rats, respectively. Whereas, HDL-cholesterol level increased by 16 and 29.4% in normal and diabetic rats, respectively, as compared with their initial values.

Sabitha *et al.* (2009) compared the lipid profile and anti oxidant status (total glutathione, glutathione peroxidase and superoxide dismutase) of persons, who were consuming coconut oil (saturated, medium chain fatty acid) with those consuming sunflower oil (polyunsaturated, long chain fatty acid). The subjects derived approximately 13 to 20% of their total calories from the oil considered and were consuming the respective oil as the predominant cooking medium for over a period of 6 yrs. They found no significant changes in lipid profile or oxidative stress parameters between coconut oil and sunflower oil consuming groups. HDL cholesterol concentrations were low for sunflower oil groups compared to coconut oil groups though, the results were not statistically significant. The findings indicate that habitual consumption of coconut and coconut oil along with normal diet has no specific role in the causation of CHD in Kerala population.

Yamamoto and Yasuoka (2010) studied the effect of two types of Welsh onion, green-leafy and white-sheath types, on hyperlipidemia in rats fed on diets high in fat and sucrose. They showed a significantly lower level of plasma cholesterol and reduced level of total lipids, triacylglycerol, and cholesterol in the liver of rats fed on the green, but not white, Welsh onion.

2.3. VIRGIN COCONUT OIL

Nevin and Rajamohan (2008) reported the following fatty acid composition for virgin coconut oil using gas chromatography (Helium as carrier gas at a flow rate of 5.4 ml/min):

Fatty acids	Percentage
Caprylic acid (8:0)	8.05
Capric acid (10:0)	5.42
Lauric acid (12:0)	45.51

Myristic acid (14:0)	19.74
Palmitic acid (16:0)	7.83
Stearic acid (18:0)	3.14
Oleic acid (18:1)	4.70
Linoleic acid (18:2)	1.88
Arachidic acid (20:0)	0.086
Gadoleic acid (20:1)	0.027
Behenic acid (22:0)	0.016
Lignoceric acid (24:0)	0.032
Other fatty acids	3.57

The total polyphenol content of virgin coconut oil was estimated to be 80 mg/100g oil.

2.3.1. Effect on consumption

Nevin and Rajamohan (2004) investigated the effect of consumption of 8% virgin coconut oil (VCO) on various lipid parameters in comparison with 8% copra oil (CO) by feeding oil to rats along with a semi-synthetic diet for a period of 45 days. They also determined whether the polyphenol fraction (PF) of VCO has any beneficial effect over the PF from other oils in preventing in vitro Cu^{2+} induced LDL oxidation and carbonyl formation. Feeding of VCO decreased the concentrations of total cholesterol, triglycerides, phospholipids, LDL and VLDL cholesterol levels and increased HDL cholesterol in serum and tissues when compared with CO. They suggested that the lower lipid levels in serum and tissues (liver, heart, and kidney) following VCO feeding might be due to the relative rate of synthesis and catabolism of these lipids and also due to the higher polyphenol content of VCO compared to CO. They also reported that polyphenols in VCO are potential antioxidants against copper induced LDL oxidation.

Isabel *et al.* (2006) evaluated the effect of phenolic content of olive oil at a daily dose of 25 ml for 3 weeks on plasma lipid levels and lipid oxidative damage in humans. They observed a decrease in the serum TAG level, a reduction in total cholesterol/ HDL ratio and an increase in the level of serum HDL. Oxidative stress markers (conjugated dienes, hydroxy fatty acids, and circulating oxidized LDL) decreased linearly with increasing phenolic content by improving the GSH/GSSG ratio.

Nevin and Rajamohan (2006) evaluated the antioxidant potential of virgin coconut oil (VCO) in comparison with coconut oil (CO) and groundnut oil when fed to rats along with a semi-synthetic diet for a period of 45 days. Either of the oils were added to the diet at 8% level. They found increased levels of antioxidant enzyme activities in tissues of VCO fed rats compared to CO. VCO also prevented peroxidation of lipids in tissues. It has been suggested that this might be due to the high content of unsaponifiable components such as vitamin E and polyphenols and also due to the differences in the absorption, transport and catabolism of the constituent fatty acids.

Nevin and Rajamohan (2008) evaluated the effect of feeding either VCO/CO/ sunflower oil (SFO) (10%) incorporated in a diet containing cholesterol (1%) for a period of 45 days on blood coagulation factors, lipid levels and in vitro oxidation of LDL and compared between the diets. They observed that feeding of VCO supplemented diet exerted a significant antithrombotic effect compared to coconut oil (CO) and the effects were comparable with SFO fed animals, which might be due to the suppression of platelet aggregation, low levels of serum cholesterol and TAG. *Invitro* studies showed that VCO prevented the formation of lipid peroxides in both erythrocyte membrane and serum LDL fraction of experimental animals. Serum levels of antioxidant vitamins were found to be higher in VCO fed animals than other groups. These properties of VCO may be attributed to the presence of biologically active unsaponifiable components viz., vitamin E, provitamin A, polyphenols and phytosterols.

Pinent (2008) reported that flavonoids increase glucose stimulated insulin secretion from islet cells, which enhances glycogenesis by the activation of the glycogen synthase system.

Zhou *et al.* (2009) evaluated the effect of flavonoids extracted from lotus (*Nelumbo nuficera*) on reducing hyperglycemia and hyperlipidemia in alloxan-induced diabetic mice. Oral administration of flavonoids (200 mg/kg) for 28 days showed significant reduction in the level of fasting blood glucose, serum total cholesterol and TAG levels. Whereas serum HDL cholesterol level were increased.

2.4. FISH OIL

Fatty acid composition of fish oil was reported by Sen *et. al.* (1997) using gas chromatography:

Fatty acids	Percentage
Myristic (14:0)	0.4
Palmitic (16:0)	1.4
Palmitoleic (16:1)	2.8
Hexadecadienoic (16:2)	0.3
Heptadecanoic (17:0)	0.4
Heptadecenoic (17:1)	0.7
Heptadecadienoic (17:2)	0.1
Stearic (18:0)	0.3
Oleic (18:1)	5.8
Linoleic (18:2)	1.1
Linolenic (18:3)	0.9
Octadecatetraenoic (18:4)	5.4
18-Isomers other	0.6

Gadoleic (20:1)	1.9
Icosatrienoic (20:3)	1.8
Arachidonic (20:4)	1.6
Eicosapentaenoic (20:5)	34.9
Behenic (22:0)	0.7
Cetolecic (22:1)	2.3
Docosatetraenoic (22:4)	0.5
Docosapentaenoic (22:5)	4.1
Docosahexaenoic (22:6)	26.4
Other fatty acids	5.6

2.4.1. Effect on consumption

Marine lipids, especially fish oil contains long-chain ω-3 polyunsaturated fatty acids (EPA and DHA), known to reduce plasma lipid level and platelet aggregation (Goodnight *et al*. 1982).

Nestel *et al*. (1984) reported that feeding fish oil (30%) for a period of 26 days lowered the levels of plasma TAG, HDL, VLDL, apo A-I and B apoprotein (apo B) in normal humans, but the effect on LDL concentration was inconsistent.

Epidemiologic studies suggest that the consumption of a diet rich in marine lipids reduce the incidence of CHD (Kromhout *et al*. 1985).

Chen *et al*. (1987) evaluated the digestion and absorption of menhaden oil, a fish oil concentrate (FOC), corn oil and oleic acid in adult male rats. The rats were administered with a single duodenal dose of an aqueous emulsion containing one of the oils. Analysis of thoracic lymph of rats administered with menhaden oil or FOC over a 24 hr period showed a lower level of cholesterol than oleic acid or corn oil and suggested that it might be due to reduced cholesterol absorption or digestion of fish oil TAG or due to higher sterol excretion.

Haug and Hostmark (1987) studied the effect of equienergetic amounts of fish oil and coconut oil on plasma lipoproteins and lipoprotein catabolizing enzymes [lipoprotein lipase (LPL) and hepatic endothelial lipase (HL)] in rats fed with purified diets containing either 22% coconut oil, coconut oil: fish oil (11:11) or 22% fish oil for 4 weeks. They observed appreciably lower levels of plasma TAG, cholesterol and phospholipid in rats fed with fish oil diet, which might be due to a fall in VLDL and HDL_2, with less consistent changes in LDL and HDL_3. Activities of the enzymes, LPL and HL were also decreased in these rats which could be an adaptive response to the low concentration of the substrates, VLDL and HDL_2, respectively for these enzymes.

Meydani *et al*. (1987) investigated the effect of age and dietary fat type on tocopherol status in mice fed semi purified diets containing 5% fish, corn or coconut oils for 6 weeks. Mice fed with fish oil diet maintained lower plasma and tissue tocopherol concentrations than those fed with corn and coconut oil diets. They suggested that the difference was not due to a loss of tocopherol prior to consumption, but rather appeared to occur during the absorption process.

Nalbone *et al*. (1988) investigated the effect of dietary salmon oil (12.5% w/w) feeding on rat heart lipid status and found that serum cholesterol, TAG, phospholipid and vitamin E concentrations were significantly lowered in rats fed with salmon oil for 2 months, whereas serum vitamin A was not significantly affected. Some intracellular enzymes, measured in the blood (e.g., transaminases) were not affected by the high fat diets, indicating that the diet induced no apparent cellular damage. In heart tissues, protein, TAG, cholesterol levels and phospholipid composition remained unchanged and no significant changes in lyso-phosphatidyl choline (PC) or lyso-phosphatidylethanolamine (PE) levels were observed. Also, the salmon oil diet produced a markedly lower n-6/n-3 ratio in both PE and PC, which might be due to the replacement of n-6 PUFA, with n-3 PUFA.

Study conducted by Garg *et al*. (1989) revealed that feeding diets containing 16% linseed oil (18:3 ω3) or 20% fish oil (primarily 20:5 ω3 and 22:6 ω3) to rats for a 4 weeks period lowered plasma cholesterol level by 13% and 33%, respectively, compared with animals fed with 18% beef tallow diet. It was noticed that when a high fat diet is fed, the ω3 fatty acid of linseed oil does not affect the concentration of liver cholesterol, whereas the ω3 fatty acids of fish oil reduced liver cholesterol level, especially cholesterol esters. This decrease in esterified cholesterol content might be due to increase in the flow of cholesterol towards bile formation.

Ventura *et al.* (1989) examined the effect of feeding fish oil, safflower oil and hydrogenated coconut oil at three different levels (5%, 10% and 20%) in rats for one month on the major process that determine the concentration of LDL in plasma. They observed that compared with equal amounts (20%) of safflower oil or coconut oil, dietary fish oil reduced plasma LDL-cholesterol levels both by reducing the rate of LDL-cholesterol production and by increasing the rate of receptor dependent LDL transport.

Clarke *et al.* (1990) examined the ability of PUFA (20% fish oil) to suppress the gene expression of FAS (Fatty acid synthase) and S14. Rats were euthanized exactly 3 hours after the meal began. They observed that PUFA were very effective at preventing the rise in FAS and SI4 mRNA and this might be attributed to the regulation of gene expression of lipogenic enzymes by PUFA.

Jenkins and Kramer (1990) studied the effect of replacing half of the lipid content (10% of dry matter) in the milk replacer (28% dry matter) with corn oil (rich in linoleic acid) or a mixture of corn oil and fish oil (rich in PUFA) either in the ratio 2:1 or 1:2 for a period of 14 days in calves. They observed decreased levels of plasma total lipids, phosphatidylcholine, and cholesteryl esters.

Lang and Davis (1990) conducted *invitro* studies in cultured rat hepatocytes to determine the effect of the two major fish oil fatty acids, EPA and DHA, each at a level of 1mM concentration, on VLDL assembly and secretion. They observed that both EPA and DHA decreased the secretion of TAG while stimulated TAG synthesis, resulting in the accumulation of intracellular TAG. Fish oil fatty acids impaired the secretion of the major core lipid of VLDL (TAG) and the major protein component (apoB) to a similar extent indicating impaired VLDL particle assembly and/or secretion. Thus the results strongly suggest that the mechanism by which fish oil fatty acids impairs TAG secretion is not direct inhibition of TAG synthesis, but through impaired VLDL assembly and/or secretion.

Parrish *et al.* (1991) reported that adipose tissue (epididymal and perirenal) is the only tissue whose mass decreased after fish oil supplementation, whereas mass of liver and spleen increased.

Tripodi *et al*. (1991) investigated the influence of feeding 8% fish oil and 6% coconut oil enriched diets for 4 weeks on the chemical composition of rat liver plasma membranes and LDL and on the binding of LDL to liver membranes. Rats fed with fish oil diet showed lower levels of total, LDL and HDL plasma cholesterol than that observed in rats fed with coconut oil and to a lesser extent lower than those of control rats. Rats on fish oil diet showed relatively low level of cholesterol and phospholipid, but a high level of TAG in LDL. Furthermore, fish oil feeding was associated with a greater concentration of n - 3 fatty acids and a lower arachidonic and linoleic acid content in LDL. The study indicated that during the period of fish oil diet, the higher binding affinity of LDL to liver plasma membranes might be partly responsible for the hypocholesterolemic action.

Banerjee *et al.* (1992) compared the effects of dietary fish oils with different n-3 PUFA compositions, on plasma lipid profiles in rats fed for a period

of 60 days. The feeding of marine Hilsa fish oil (10%) for sixty days decreased the levels of cholesterol, TAG and phospholipid in rat plasma. A similar treatment with chital oil (Fresh water fish) diet (10%) elevated the plasma cholesterol level while TAG and phospholipid contents remained unaltered. The study strongly suggests that the hypolipidemic effect depends on the composition of the n-3 PUFA rather than on the total n-3 PUFA content of the dietary fish oil.

Studies conducted by Rustan *et al*. (1993) revealed that high intake of fish oil derived very long-chain n-3 PUFAs (6.5%), EPA and DHA for 6-8 weeks lowered nonfasting plasma concentrations of TAG, phospholipids, unesterified fatty acids and glycerol in rats compared to a diet containing mostly saturated and monounsaturated fat. The reduced level of plasma unesterified fatty acids is suggested to be due to decreased mobilization of fatty acids from adipose tissue or due to an increased removal of fatty acids from the circulation. Liver and muscle content of glycogen were found to be decreased in the n-3 fatty acid fed rats. Increased LPL activity in muscle tissue was also observed, which indicated increased fatty acid utilization in these tissues, thereby lowering the level of plasma TAG.

Willumsen *et al*. (1993) showed that DHA at a dose of 1000mg/kg b.w/day for 10 days does not inhibit the synthesis and secretion of TAG in the liver of rats. In contrast to DHA, EPA administration showed a hypotriglyceridemic effect. Both EPA and DHA increased peroxisomal β-oxidation and fatty acyl-CoA oxidase activity. EPA also stimulated the activities of carnitine palmitoyltransferase and diacylglycerol acyltransferase but phosphatidate phosphohydrolase activity was unaffected. They suggested that increased mitochondrial oxidation of fatty acids and thereby decreased availability of nonesterified fatty acids may be the mechanism behind the hypotriglyceridaemic effect of EPA. It was also noted that stimulation of peroxisomal β-oxidation by n-3 fatty acids is not sufficient to decrease the serum levels of TAG.

Smit *et al*. (1994) investigated the effect of feeding diets incorporated with fish oil or corn oil (9% w/w) for 2 weeks on plasma lipids and biliary lipid composition, on the day-night variation in biliary cholesterol output and on the capacity of taurochenodeoxycholic acid (TCDC) and bilirubin ditaurate (BDT) to stimulate and to inhibit, respectively, biliary cholesterol output. They found that intravenous administration of the bile acid (15 μmol/kg) induced the secretion of 2-3 times as much cholesterol in fish oil than in corn oil fed rats. Whereas, administration of BDT (30 μmol/kg), an inhibitor of bile acid-induced biliary lipid secretion, reduced cholesterol output in both groups by about 50% while bile acid output remained unchanged. Also, cholesterol secretion relative to that of bile acids in fish oil fed rats was even more increased during the night. They suggested that dietary fish oil increases the disposition of cholesterol into bile by potentiating bile acid dependent cholesterol secretion, presumably by facilitating the recruitment of bile destined cholesterol.

Rambjor *et al.* (1996) conducted a study in healthy volunteers for 3 weeks using 91% pure EPA, 83% pure DHA and fish oil concentrate (FOC) containing either EPA and DHA at 41 % and 23% respectively on fasting lipids, lipoproteins and phospholipid fatty acid composition. The target n-3 fatty acid intake was at a level of 3g/day and that of FOC at a level of 5g/day. They found that on DHA supplementation, EPA levels increased to a small but significant extent, suggesting that some retroconversion may have occurred. However, EPA supplementation did not raise DHA levels. Also FOC and EPA produced significant decrease in both serum TAG and VLDL cholesterol levels and increase in serum LDL cholesterol levels. DHA supplementation did not affect any of these including HDL levels. Thus it is concluded that EPA is primarily responsible for TAG-lowering and LDL raising effects of fish oil.

Demonty *et al.* (1998) fed Sprague-Dawley rats with purified diets varying in both protein (20%) and lipid (11%) source consisting of either casein–menhaden oil, casein–coconut oil, soy protein–menhaden oil, soy protein–coconut

oil, cod protein–menhaden oil, or cod protein–coconut oil for 28 days to verify the independent and interactive effects of dietary proteins and lipids on serum and hepatic lipids. Coconut oil diets provoked higher levels of total and HDL cholesterol than menhaden oil diets. Casein and cod protein induced higher hepatic cholesterol concentrations than soy protein. Neither an independent lipid effect nor a protein–lipid interaction was observed on hepatic cholesterol concentrations. The results also showed that there are interactions between dietary proteins and lipids in the regulation of serum TAG levels in the rat. Indeed, soy protein enhanced the hypotriglyceridaemic effect of fish oil, whereas casein and to a greater extent cod protein dampened it. They suggested that dietary proteins can modulate the effects of fish oil on serum TAG concentrations through hepatic cholesterol and TAG concentrations.

Mortensen *et al*. (1998) compared the effects of fish oil and olive oil on the development of atherosclerosis in Watanabe heritable hyperlipidaemic (WHHL) rabbits by feeding a daily dose of 1.5 ml/ kg body weight for 4 weeks. Compared with olive oil, fish oil significantly decreased plasma cholesterol and TAG levels. Fish oil administered group also showed significantly lower levels of cholesterol and TAGs were in IDL and VLDL when compared with the olive oil group, whereas no significant difference was noted in the levels of LDL-cholesterol and LDL-TAGs between groups. Moreover, significantly higher LDL oxidation rate was observed in the fish oil group when compared with that of olive oil group.

Vlijmen *et al.* (1998) tested the effects of dietary fish oil (0, 3 and 6% w/w) on serum lipids and VLDL kinetics under highly standardized conditions using hyperlipidemic apolipoprotein (APO) E*3-Leiden transgenic mice. Following 4 weeks of fish oil (3%) administration, significant dose dependent decrease was observed in serum cholesterol levels. Level of plasma TAG decreased by feeding 3% fish oil diet while no further decrease was observed at 6% level. LDL and HDL cholesterol levels were not affected. It has been

suggested that fish oil decreased the production rate of hepatic VLDL TAG and increased the rate of catabolism of VLDL-apoB resulting in decreased level of serum VLDL.

Chitra *et al.* (2000) administered fish oil (Menhaden oil) orally, to male albino rats at a dose of 0.5 ml/kg b.w/day for 30 and 90 days, to study the effect in cigarette smoke (2 hr/day) induced dyslipidemia in rats. They found that total cholesterol and TAG level in serum and tissues (heart and lungs) were significantly increased in rats exposed to cigarette smoke for 30 and 90 days. Co-treatment with fish oil lowered serum and tissue cholesterol and TAG levels. They suggested that eicosanoids especially, prostaglandins in fish oil, increased the level of cAMP in platelets, lungs and adipose tissue which in turn decreased lipolysis at the adenylate cyclase site, acting through a Gi protein.

Nestel (2000) reviewed that fish oil supplementation increased HDL_2-cholesterol level, reduced TAG rich lipoprotein and postprandial lipaemia. In contrast, LDL-cholesterol level increased and showed an increased oxidizability, which could be compensated with vitamin E supplementation.

The n-3 PUFAs play a crucial role as “fuel partitioners,” in that they direct fatty acids towards oxidation rather than storage as TAG. They act by upregulating the expression of genes encoding proteins involved in fatty acid oxidation while downregulating genes encoding proteins of lipid synthesis (Clarke, 2001).

Ikeda *et al.* (2001) in a series of studies in rats, examined the effect of fish oil on the absorption of TAG, activities of LPL and hepatic triacylglycerol lipase (HTAGL), metabolism of chylomicrons (CM) and CM remnants and the secretion of TAG from the liver to evaluate the mechanism of suppression of postprandial hypertriglyceridaemia. They found that the activities of LPL in adipose tissue and heart were increased and HTAGL decreased and did not

influence the absorption of dietary fat and the clearance rate of CM and CM remnants. Also feeding of fish oil for 3 weeks suppressed postprandial hypertriglyceridemia and suggested that FO reduced TAG secretion from liver.

Sachan *et al*. (2002) compared the effects of 10% corn oil, safflower oil, menhaden fish oil and palm oil on carnitine status and ethanol metabolism in rats for 8 weeks. Fish oil and palm oil caused a reduction in ethanol metabolism and lowered ethanol clearance rate. But, corn oil and safflower oil hastened ethanol clearance indicating faster ethanol metabolism and greater liver damage because of oleic and linoleic acids. The effect of dietary fats in carnitine species in plasma, urine and liver was varied and was not related to the change in ethanol clearance. However, the liver carnitine content was higher in the palm oil group. They also found that palm oil and fish oil fed animals showed significantly lower acid insoluble acyl carnitine (AIAC) compared to corn oil and safflower oil fed animals after 8 weeks of treatment. FO fed group showed the lowest AIAC and total carnitine levels.

Caso *et al*. (2003) showed that substituting fish oil (14g/100g lipids) for standard oils (a mixture of oils from vegetable and animal origins) for a period of 3 weeks protected rats from visceral fat hypertrophy, hypertriglyceridemia and hyperglycemia. Rats fed with fish oil diet showed greater lipolysis in adipocytes, lower Fatty acid synthase (FAS) activity in liver, and about 2.2 fold higher LPL activity in adipose tissues. The decrease in visceral fat in rats fed fish oil was attributed to decreased plasma TAG concentration and/or increased lipid mobilization rather than to reduced lipid storage.

Ahmed *et al.* (2006) studied the effect of 4 weeks feeding of 10% fish oil, soybean oil and palm oil on the lipid profile of experimentally induced hypercholesterolemic rats (fed with 1% cholesterol for 14 days) for 28 days. The fish oil feeding caused significant reduction in serum cholesterol, LDL and an increase in HDL level. There was no significant difference in serum cholesterol

level between the rats fed with 10% soybean oil and 10% palm oil. Liver cholesterol level was also significantly reduced in the fish oil group.

Maree *et al*. (2009) evaluated the role of 10% dietary fish and sesame oils as protective agents or for treatment of hyperlipidemia produced by a 20% coconut oil based high fat diet in rats fed for a period of 12 weeks. Rats fed on coconut oil showed significant elevation in the levels of serum total cholesterol, LDL-cholesterol and atherogenic factors while HDL-cholesterol level was significantly decreased. Histological examination revealed large lipid depositions in livers of rats fed with high fat diet. They observed that supplementation of diet with fish oil or a mixture of fish/ sesame oil lowered blood cholesterol, LDL-cholesterol and elevated HDL-cholesterol level. In addition, these oils prevented deposition of lipids in nephrocytes and hepatocytes.

Park and Park (2009) investigated the effect of feeding 10% fish oil, shortening or soybean oil for 4 weekss in rats. They observed significantly lower levels for Total cholesterol, TAG, and C-reactive protein in the fish oil than in soybean oil and shortening groups. LDL cholesterol levels were significantly ($P<0.001$) lower in the fish oil and shortening groups than in soybean oil group. Also rats fed fish oil showed significantly lower abdominal fat weight, but significantly higher liver and kidney weights, as compared with those fed soybean oil or shortening.

2.5. FATTY ACIDS AND TRIACYLGLYCEROL (TAG)

2.5.1. Medium and long chain fatty acids and triacylglycerol (TAG)

Free medium-chain fatty acids (MCFAs) are absorbed readily into gastrointestinal cells, which diffuse rapidly into the portal circulation and after reaching the liver, these are oxidized for energy purpose whereas, long chain fatty

acids (LCFAs) require the detergent action of bile to enter the intestinal cell (Clark and Holt, 1968)

Medium chain triacylglycerols (MCTs) are readily hydrolyzed by lingual and gastric lipases to release MCFAs while Long chain TAG (LCT) requires intestinal lipase for cleavage (Bezard and Bugaut, 1986).

Cater *et al*. (1997) conducted studies in humans for a period of 3 weeks and compared the effects of 43% MCT with equal amount of two different fats, palm oil, rich in palmitic acid (long chain fatty acid known to raise serum cholesterol concentration) and sunflower oil, rich in oleic acid (a long-chain fatty acid known to have neutral effect on serum cholesterol concentration). They found that diet containing MCT-oil significantly increased serum TAG level compared to the other two diets. MCT-oil diet also increased total and LDL-cholesterol levels, which were similar to that of palm oil diet, but significantly higher than that of sunflower oil diet. There was no difference in HDL cholesterol concentrations between the diets.

Lal *et al.* (1999) made an attempt to find out the relationship between fatty acid chain length, cholecystokinin (CCK) secretion and proximal and distal gastric motor function in healthy volunteers and observed that fatty acids of 11 carbon chains and below that could not increase plasma CCK concentration, whereas long chain fatty acids could. Though, the finding could not support the role of CCK in mediating the satiating effect of MCT, it showed that the human proximal gut is able to differentiate between fatty acid molecules.

Tsuji *et al*. (2001) investigated the effect of long-term (12 weeks) ingestion of dietary MCT and LCT at the level of 60g/day, on body weight and fat in humans. They observed that in individuals with BMI > = 23 kg/m^2, body weight and body fat in the MCT group were significantly lower than those in the LCT (blended rapeseed oil and soybean oil) group.

Pierre *et al*. (2002) reported that replacing dietary LCT by MCT for 4 weeks caused a rise in energy expenditure, a depression of food intake, lowered body fat mass and decreased the size of fat depots in 24 healthy over-weight men.

A study conducted by St-Onge *et al*. (2003) revealed that a functional oil rich in MCT (64.7%), when consumed as part of a strictly controlled targeted weight maintenance diet for 28 days, caused greater loss of adipose tissue stores compared with a diet rich in LCT in human subjects. They suggested that this change in total adiposity may be due to enhanced energy expenditure through fat oxidation and greater fecal fat excretion.

Pierre *et al*. (2008) reported that incorporating either 18-24g/day of MCT oil in a free-living weight-loss diet for 16 weeks led to greater losses of body weight and fat mass compared to consumption of an equivalent amount of LCT oil in 49 overweight men and women (aged 19-50 yrs).

Takeuchi *et al*. (2008) reviewed the physiological function of MCFAs and medium and long chain TAGs (MLCTs). They reported that both MCT and MLCT are rapidly digested, absorbed and suppresses body fat accumulation by increasing energy expenditure.

Liu *et al*. (2009) conducted experiments in individuals using MLCT diet and LCT diet. They observed that consumption of 25-30g of MLCT for 8 weeks reduced body weight, body fat, level of serum TAG and improved apolipoprotein metabolism in males, while females showed a similar response in both MLCT and LCT oil groups. They suggested that the different effects of MLCT and LCT consumption on lipid metabolism in men and women may be due to differences in energy intake between men and women or due to a hormonal effect. Oxidation of fatty acids stimulated by intake of MCFAs is suggested to be the most possible mechanism for lowering TAG after the consumption of MLCT oil.

Yang *et al*. (2009) reported that 0.1% octanoate and decanoate, 8-carbon and 10-carbon MCFAs, decreased adipogenesis in 3T3-L1 mouse embryo preadipocytes when treated with standard hormonal cocktail (isomethylbutylxanthine, dexamethasone and insulin), but increased adipogenesis in a dose-dependent manner (with decanoate being more effective) when treated with basal media containing 10% foetal bovine serum (FBS). Addition of dexamethasone to basal medium with either octanoate or decanoate further increased adipogenesis. The opposite effects of octanoate and decanoate on adipogenesis with and without standard hormonal cocktail was attributed to the possibility that MCFA can act as either peroxisome proliferator activated receptor (PPARγ) agonist, an adipocyte specific transcription factor, or PPARγ antagonists, depending on the concentration of exogenous hormones.

2.5.2. Unsaturated fatty acids and TAG

Supplementation of 10% PUFA to a fat-free diet in rats over a period of 3 days suppressed fatty acid synthesis activities of acetyl Co-A carboxylase and fatty acid synthetase. The activity of glycolytic enzymes like glucokinase, phosphofructokinase and pyruvate kinase was not affected by PUFA (Toussant *et al*. 1981).

Etherton *et al*. (1984) demonstrated an effect of dietary fat saturation on plasma lipids and hepatic lipoprotein production in weaned male rats fed with diets containing 10% safflower oil, corn oil, olive oil or palm oil for 2 weeks. They observed decreased levels of cholesterol and TAG in serum of rats fed with safflower and corn oil diets compared with olive oil diet and this was the result of a lower chylomicron cholesterol concentration. Hepatic HDL cholesterol production and the ratio of cholesterol to protein in both hepatic HDL and VLDL fraction was significantly higher in rats on safflower oil or corn oil diets compared with rats fed with palm oil diet.

Mattson and Grundy (1985) conducted a 4 weeks study in 20 patients and compared the effects of monounsaturated (oleic) and polyunsaturated (linoleic) fatty acids as high oleic safflower oil and high linoleic safflower oil respectively, when substituted for saturated (palmitic) acids as palm oil, which supplied 40% of calories as fat, on plasma lipoproteins. They found that compared to the saturated diet, both unsaturated fatty acids caused significant reduction in total cholesterol and LDL-cholesterol, though there was no significant variation between the two fats. No changes overall were found to be produced by either unsaturated fat in levels of TAG, VLDL-cholesterol, or HDL-cholesterol. However, HDL cholesterol concentration was low in the saturated diet.

Spady and Dietschy (1985) conducted studies in hamster for 30 days to test the effect of feeding cholesterol (0.12%) alone or cholesterol plus polyunsaturated or saturated TAGs on receptor-dependent and receptor-independent hepatic LDL uptake. They observed that receptor-mediated LDL transport was suppressed ~30% by cholesterol feeding alone (0.12%) and this was unaffected by the addition of polyunsaturated TAGs, as 20% safflower oil to the diet. In contrast, receptor-dependent uptake was suppressed ~90% by the intake of saturated TAGs (as 20% coconut oil). Moreover, intake of saturated lipids also caused a significant increase in the level of plasma LDL-cholesterol and decrease in liver cholesteryl esters.

Loscalzo *et al*. (1987) conducted invitro studies to examine the effect of linoleate and oleate (cis-unsaturated fatty acids) incorporation in peripheral blood mononuclear cell membranes on the physical properties of the membrane, its LDL uptake and degradation. They found that membrane enrichment with cis-unsaturated fatty acids increased the rate of LDL degradation in freshly isolated mononuclear cells compared to enrichment with stearate.

Brinton *et al*. (1990) examined the effect of mixed dietary changes on the metabolism of the major HDL apo A-I and A-II and explored the metabolic basis

of individual variability in HDL response to dietary change during a 2 weeks experimental period. Compared to a diet low in saturated fat, total fat and cholesterol to a diet high in these lipids, HDL-cholesterol and apo A-I levels decreased in all subjects, although wide variation was observed between individuals. Apo A-I fractional catabolic rate (FCR) increased and a fall in the apo A-I transport rate (TR) was noted. They suggested that diet-induced changes in HDL-cholesterol level positively correlate with apo A-I TR and variation in HDL-cholesterol level between individuals on a given diet positively correlate with the variation in apo A-I FCR.

Khosla and Hayes (1992) compared the effects of dietary saturated (16:0), MUFAs (18:1) and PUFAs (18:2) on plasma lipoprotein metabolism in cebus and rhesus monkeys fed with cholesterol-free diets providing 40% of energy as fat for 6 weeks. They found that in the absence of exogenous cholesterol, dietary 18: 1 and 16: 0 exerted similar effects on total cholesterol in both cebus and rhesus monkeys, whereas dietary 18:2 was hypocholesterolaemic only in cebus monkeys, attributed to a decreased HDL-cholesterol concentration due to a decrease in apo A-1 production. Intake of diet containing saturated fat increased plasma TAG concentration 34% and 63% higher than that of MUFA and PUFA, respectively.

Mensink and Katan (1992) reviewed that saturated fatty acids when compared to unsaturated fatty acids increase the serum HDL concentration, which has been associated with increased LCAT activity.

Brousseau *et al.* (1993) reported that substitution of dietary saturated fatty acids (18%) with either MUFAs (6%) or PUFAs (6%) for three 13-weeks periods in cynomolgus monkeys significantly reduced plasma total cholesterol, HDL cholesterol and apo A-l and B concentrations, whereas PUFA diet significantly decreased both plasma VLDL and LDL cholesterol levels relative to the high saturated fat diet. They suggested that a decreased production of LDL apo B was responsible for the reduced level of apo B observed in the MUFA and PUFA fed

groups. However, enhanced LDL and apo B catabolism accounted for the greater reductions in VLDL and LDL cholesterol and apo B concentrations in PUFA diet fed groups.

Nydahl *et al.* (1994) compared the effect of diets enriched with MUFAs as olive oil (15%) and PUFAs as corn oil (11%) on the serum lipoprotein composition in patients with hyperlipidaemia over a 7 week period. They observed that both MUFA and PUFA enriched diets seemed to be equally effective in reducing plasma total, HDL and LDL cholesterol concentrations.

Christon *et al*. (1995) investigated the effects of dietary PUFA on the age dependent changes (at 6 months and 24 months) in liver glutathione antioxidant system in male Wistar rats. They observed a reduction in the level of liver GSH and an increase in oxidized glutathione (GSSG) concentration with age. The study strongly suggested that dietary PUFA affect the cellular glutathione dependent antioxidant system and may modify the age related changes in metabolic reactions.

Aro *et al.* (1997) tested the effect of diets containing stearic acid (9.3%), trans fatty acids (8.7%) and dairy fat (32.2-33.9%) on serum lipoproteins in healthy subjects for 5 weeks. Compared to dairy fat diet, both diets with stearic acid and trans fatty acid decreased serum total cholesterol level, but a significant reduction was observed in the level of HDL cholesterol and apo A-I in trans fatty acid diet individuals than stearic acid diet. Stearic acid diet also reduced significantly the level of LDL cholesterol and apo B. The trans fatty acid diet increased the ratio of LDL to HDL cholesterol and of apo B to apo A-I more than that of dairy fat diet but the stearic acid diet had no effect. Lp (a) concentration was also found to be increased with both diets, significantly more with trans fatty acids than with stearic acid.

Monounsaturated fatty acids like oleate (C18:1, n-9) or saturated fatty acids like palmitate (C16:0) and medium-chain fatty acids [as present in coconut oil (CO)] do not inhibit either the activities or the expression of the lipogenic enzymes (Clarke and Abraham, 1998).

Wang *et al*. (1998) studied the effect of feeding of dietary TAG of varying fatty acid composition 1 week at 753 kJ/kg/day with 51% of energy from fat on small intestinal and hepatic apolipoprotein expression, as well as serum lipid and apolipoprotein concentrations in newborn swine. They found that feeding of intermediate chain saturated triglyceride (ICST), as coconut oil (46% 12:0 and 19% 14:0 fatty acids), increased serum total cholesterol, TAG, HDL cholesterol, and apo A-I concentrations and hepatic expression of apo A-I and apo C-III mRNA, compared with feeding of MCT, as MCT oil (75% 8:0 and 24% 10:0 fatty acids), or long chain polyunsaturated TAG (LCPUT), as safflower oil (77% 18:2 and 13% 18:1 fatty acids) . They suggested that increased hepatic apo A-I expression might contribute to the higher serum HDL and apo A-I concentrations and high serum TAG level might be due to the inhibition of catabolism of TAG-rich lipoproteins, by apo C-III, when expression is increased in hepatic tissue.

Clarke (2001) reviewed that n-3 PUFAs play a crucial role as “fuel partitioners,” in that they direct fatty acids towards oxidation rather than storage as triacylglycerol. They act by upregulating the expression of genes encoding proteins involved in fatty acid oxidation while downregulating genes encoding proteins of lipid synthesis.

Summers *et al*. (2002) reported that substituting dietary saturated fatty acid with PUFA for a time period of 10 weeks reduced energy and fat intake in subjects, although body weights did not change. Insulin sensitivity and plasma LDL-cholesterol concentrations improved with the diet rich in PUFA compared with the diet rich in saturated fatty acids. Also a decrease in abdominal subcutaneous fat area was observed. They concluded that this dietary

manipulation improved insulin sensitivity reducing the risk of Type II diabetes mellitus.

Hays *et al.* (2003) conducted a 6 week trial to determine whether a diet of high saturated fat and avoidance of starch (HSF-SA) results in weight loss without adverse effects on serum lipids in obese nondiabetic patients with atherosclerotic cardiovascular disease (ASCVD). The individuals were instructed to consume one half of all calories as saturated fat, primarily as red meat and cheese. The diet resulted in decrease in body weight, calculated body fat, fasting serum glucose, insulin and TAG levels. An NMR spectroscopic assay of serum lipids did not show any effect on the levels of serum total cholesterol, LDL and HDL.

Vecera *et al.* (2003) compared the effect of a diet incorporated with 1% cholesterol containing either 10% currant oil diet (COD), rich in PUFAn-3 and PUFAn-6, or lard fat diet, rich in saturated and monounsaturated fatty acids, on antioxidant parameters, lipoprotein profile and liver lipids in rats. After 3 weeks of feeding experiment, COD induced a significant decrease in blood GSH and an increase in Cu2+ induced oxidizability of serum lipids, but did not affect liver GSH and lipoperoxidation of liver microsomes. They concluded that COD when substituted for lard fat in a high cholesterol diet, partly exerts a positive effect on liver lipid metabolism, but partly has adverse effects on antioxidant status, especially in the blood.

Benson and Devi (2009) evaluated the influence of ω 6 (10% sunflower oil) and ω 3 (10% mustard oil) PUFA diets on lipid profile and endogenous antioxidant enzymes in normal and stressed rats for 45 days. The sunflower oil treated normal rats showed significantly reduced levels of total cholesterol, HDL-cholesterol, and catalase activity thereby significantly increased the atherogenic index (AI) and lipid peroxidation, the effect being worse in stressed rats. However, mustard oil diet increased superoxide dismutase and decreased lipid peroxidation significantly in normal rats. This effect of mustard oil was attributed

to the ability of ω 3 fatty acids to inhibit inflammatory pathways and suppress the expression of large number of genes related to lipid metabolism. The results showed that not just PUFA, but the type of PUFA present in the dietary oil is important.

2.6. OIL CONSUMPTION AND OXIDATIVE STATUS

Studies conducted by Labbe *et al*. (1991) showed that diets high in (n-3) fatty acids for 16 weeks, especially menhaden oil (25% or 50%), reduced serum total cholesterol, which was primarily due to a decrease in HDL cholesterol in wistar rats. However, their consumption resulted in increased tissue thiobarbituric acid reacting substances (TBARS), showing increased lipid peroxidation, which might be due to increased incorporation of (n-3) fatty acid in tissues (liver and heart) and decreased activity of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSHPx).

Meydani *et al*. (1991) evaluated the effect of long term fish oil supplementation on vitamin E status and lipid peroxidation in 15 young and 10 older women who received daily six capsules of fish oil containing a total of 1,680 mg EPA, 720 mg DHA, 600 mg other fatty acids, and 6 IU vitamin E for 3 months. They found that all women showed a significant increase in plasma lipid peroxide during the second month of supplementation, but after the second month, older women had significantly higher lipid peroxide levels than young women. Long term fish oil supplementation was found beneficial in reducing plasma total TAG, but susceptibility of plasma lipids to free radical attack was potentiated. Fish oil also caused a significant reduction in plasma vitamin E. Decreased hepatic secretion of VLDL has been considered to be the major contributing factor for the hypotriglyceridaemic effect of fish oil.

Gonzalez *et al*. (1992) reported that addition of a synthetic antioxidant, butylated hydroxytoluene, 0.02 g/100 g oil, to diets with high levels of PUFA

(19% fish oil) (recommended by American Institute of Nutrition (AIN)) was found to be insufficient to completely inhibit oxidative deterioration of such diets. Daily feeding of this diet to mice for a period of 4 weeks caused an accumulation of lipid peroxidation products in certain organs (e.g., heart, skeletal muscle and mammary glands) and in the carcass.

Ibrahim *et al*. (1997) investigated the effects of dietary lipid, vitamin E and iron on lipid and protein oxidation and antioxidant status in mouse liver in a 4 weeks study. Mice fed with fish oil (8%), showed significantly higher hepatic concentration of TBARS and conjugated dienes than those receiving lard. The hepatic levels of TBARS, conjugated dienes and protein bound carbonyls were lower in the fish oil fed group receiving vitamin E than in those without supplementation. Hepatic GSH level was significantly higher in mice fed with fish oil diet than lard diet, which was suggested to be due to an adaptive response to increased oxidative stress.

Sen *et al.* (1997) conducted studies in rats for a period of 8 weeks with diet supplemented with α-tocopherol (vitamin E), fish oil and soy oil on resting and exercise induced oxidative stress and found that fish oil (1 g/kg b.w/day) induced oxidative damage of lipids in a tissue specific manner. Fish oil and vitamin E (500 mg/kg b.w) (FOVE) supplementation decreased tissue oxidative stress, as shown by both TBARS and protein carbonyl levels, than that of non-vitamin E supplemented animals, but hepatic lipid peroxidation remained higher than in the soy oil and vitamin E (SOVE) supplemented rats.

Vaagenes et al. (1998) observed that increased peroxisomal β-oxidation primarily seemed to cause oxidative stress in rats fed with PUFA. However, the amount of accumulated PUFA in the cell membranes is the critical parameter. It has been shown that administration of low doses of lipid lowering Eicosa pentaenoic acid (EPA) derivatives (250mg/d/kg b.w) for 5 days did not affect lipid peroxidation. High doses of EPA and DHA (1500mg/d/kg b.w)

administration for 7 days, however, elevated the levels of malondialdehyde (MDA) in plasma and liver.

Mete *et al.* (1999) reported that fish oil (10%) fed rats for a period of 10 weeks produced higher levels of plasma and liver MDA, with more accumulation in liver tissues than in plasma and lowered the levels of liver glutathione peroxidase (GSH-Px) and catalase activity compared with the soybean oil (10%) fed rats. Fish oil also decreased erythrocyte GSH concentration by decreasing Glucose-6-phosphate dehydrogenase (G6PD) activity. They observed that higher doses of vitamin E when fed together with fish oil decreased MDA production and also improved the antioxidant status. A positive correlation was found between dietary vitamin E and plasma vitamin C, erythrocyte GSH and liver GSH-Px.

Xi and Chen (2000) studied the effects of dietary fish oil (17%) on major endogenous antioxidant defense parameters in tissues of mice with murine AIDS (MAIDS). They showed that dietary fish oil supplementation for 4 weekss prevented the suppression or stimulated most of the antioxidant mechanism indicating possible protective effects against oxidative stress resulting from viral infection. They suggested that fish oil may have a therapeutic potential as an adjunct therapy for human AIDS.

Primary products of lipid peroxidation can undergo carbon–carbon bond cleavage via alkoxyl radicals in the presence of transition metals, giving rise to the formation of short-chain, unesterified aldehydes (Kawai *et al.,* 2003).

Godwin and Prabhu (2006) studied the level of TBARS in various fish oils available in the market with and without added Vitamin E. They observed that the peroxide levels in almost all the products available in the market were abnormally high irrespective of their Vitamin E content. This was suggested to be due to the inefficient methods used for processing and storage of fish oils. Addition of

vitamin E was found to have a significant effect in lowering the rate of peroxidation of fish oil during thermal stress (heated to food frying temperature of 180ºC), showing that association of antioxidants with ω-3 fatty acids lowers the rate of lipid peroxidation.

3. MATERIALS AND METHODS

3.1. PROCUREMENT OF OILS

1. Commercial Coconut oil (Copra oil/ RBD coconut oil): Procured from Kerala Agricultural University.
2. Commercial virgin coconut oil: RUBCO Nutri-ko virgin coconut oil was procured locally.
3. Commercial shark liver oil: Procured locally from market.
4. Seasoned coconut oil: Was prepared in laboratory. A small portion of copra oil was heated in a pan and added mustard seeds (10%). When the seeds sizzled, added chopped small onion/ red onion (*Allium oschaninii*) (5%) and fried until the colour became golden brown. Then added the remaining oil followed by turmeric powder (2.5%) and curry leaves (10%). Mixed well and allowed to cool to room temperature. Then strained the oil through a muslin cloth and was used for the study.

3.2. EXPERIMENTAL ANIMALS

The study was conducted in 30 adult male Wistar rats weighing 180-220g. The rats were purchased from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The animals were housed in appropriate cages in a well ventilated experimental animal room under 12:12 hr LD cycle at 22 to 28^{o} C and 45 to 55% relative humidity with free access to standard rat pellet diet and drinking water. An acclimatization period of one week was allowed before commencement of the experiment. The experiment was conducted for a period of 90 days.

3.3. EXPERIMENTAL DESIGN

The animals were randomly divided into five groups comprising 6 animals each and were provided with *ad libitum* feed and water. Group 1 contained rats without any treatment (normal control). All other groups were administered with various oils as follows:

GROUPS	TREATMENTS
G1	Normal Control (NC)
G2	Copra oil (CO)
G3	Seasoned coconut oil (SCO)
G4	Virgin coconut oil (VCO)
G5	Fish oil (FO)

3.4. FIXATION OF DOSE AND ADMINISTRATION OF OILS

Dose was fixed based on per capita world average consumption level (17.8 kg/head/year), consumption level of developed western world (44 to 48 kg/head/year) and the total coconut oil consumption in kerala (free oil + oil derived from kernel), which comes around 14 kg/head/year (Rajamohan, 2004). A dose of 30 kg/head/year was fixed, which stands in between per capita world average consumption and consumption of developed western world, also which is much higher than the per capita coconut oil consumption in kerala. Human dose was converted to rat dose, 16.4 g/kg body weight (b.w.) per day and was administered orally using an orogastric tube in divided doses at morning and evening.

3.5. BODY WEIGHT

Weight of the animals was recorded just before oil administration (day 0) and later at 45 and 90 days after administration.

3.6. COLLECTION OF BIOLOGICAL MATERIALS

3.6.1. Collection of blood and separation of serum

Blood samples were collected from all rats just before oil administration (day 0), and later at 45 and 90 days after administration. Blood was collected from the retroorbital plexus under mild ether anesthesia, using heparinised capillary tubes, into sterile microfuge tubes. The tubes were kept undisturbed for one hour at room temperature and then transferred to 4°C for clot retraction. After 30 minutes the tubes were centrifuged at 30 x g for 10 minutes at 15ºC for separating the serum.

3.6.2. Collection of liver and heart

Liver and heart were collected after euthanizing the animals under ether anesthesia. The organs were washed in ice cold saline to remove blood clots and kept in chilled saline. Weight of liver and heart was recorded.

3.7. BIOCHEMICAL PARAMETERS ANALYSED

3.7.1. Estimation of serum metabolites

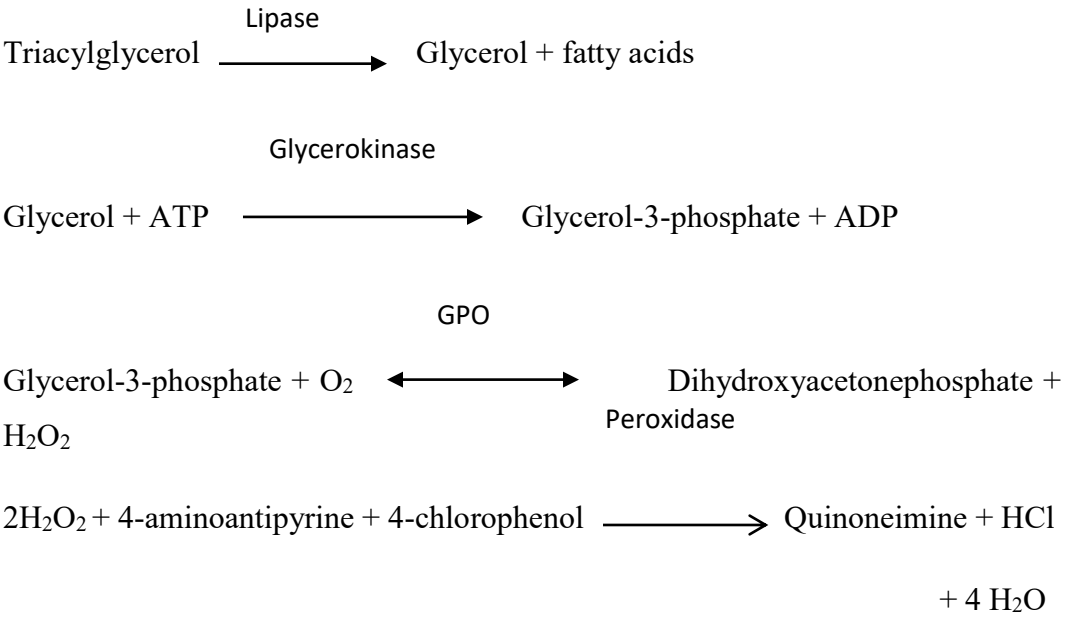
Serum metabolites were estimated using commercial kits. The concentrations of various metabolites were directly measured using semiautomatic blood analyzer, Microlab – 200 (M/s E. Merck India Limited, Mumbai).

3.7.1.1. Triacylglycerol (TAG)

Concentration of serum triacylglycerol was estimated by GPO-POD method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Photometric determination of triacylglycerol involves enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.



Reagents:

Reagent 1:	Good's buffer, pH 7.2	50 mmol/L
	4-chlorophenol	4 mmol/L
	ATP	2 mmol/L
	Mg^{2+}	15 mmol/L
	Glycerokinase	≥0.4 kU/L
	Peroxidase	≥2 kU/L

	Lipoprotein lipase	≥2 kU/L
	4-aminoantipyrine	0.5 mmol/L
	Glycerol-3-phosphate oxidase (GPO)	≥1.5 kU/lL
Standard:	Triacylglycerol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl
Mixed, incubated for 5 minutes at 37ºC. Absorbance was read against the blank at 546 nm within 60 minutes.		

Calculation:

$$\text{Triacylglycerol in mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard.}$$

3.7.1.2. Total lipids (TL)

Concentration of serum total lipids was estimated using Labkit (M/s E. Merck India Limited, Mumbai).

Principle:

Unsaturated lipids react with sulphuric acid to form carbonium ions. In a second step the carbonium ions react with phosphovainilline to give a pink colour.

The intensity of the color formed is proportional to the total lipids concentration in the sample.

Reagents:

Reagent	Phosphovainilline	235 mmol/L.
Standard	Total Lipids aqueous primary standard	750 mg/dl.
Additional reagent	Concentrated Sulphuric acid	

Procedure:

1. Assay Conditions

Wavelength:	520 (490-550) nm.
Cuvette:	1 cm light path.
Temperature	37ºC.

Adjusted the instrument to zero with distilled water.

2. Preparation of acid digest:

Labeled two cuvettes into standard and sample and proceeded as follows:

	Standard	Sample
H_2SO_4	2.5 ml	2.5ml
Standard	100 µl	-
Sample	-	100 µl

3. Mixed thoroughly using a mechanical stirrer.

4. Incubated for 10 minutes in a boiling water bath (100ºC).

	Blank	Standard	Sample
R	1.0 ml	1.0 ml	1.0 ml
Sample acid digest	-	-	50 µl
Calibrator acid digest	-	50 µl	-

5. Cooled in iced water and proceeded as follows:

6. Mixed thoroughly using a mechanical stirrer.

7. Incubated for 15 minutes at 37ºC.

8. The absorbance (A) of the samples and calibrator was read against the Blank. The colour is stable for at least 1 hour.

Calculation:

$$\text{Total Lipids (mg/dl.)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times 750 \text{ (Calibrator conc.)}$$

3.7.1.3. Total Cholesterol (TC)

Concentration of serum cholesterol was estimated by Cholesterol oxidase phenol amino antipyrine (CHOD-PAP) method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

Principle:

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent oxidation by cholesterol oxidase, H_2O_2 is liberated. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase.

Reagents:

Reagent 1:	PIPE's buffer, pH 7.5	99 mmol/L
	Salicylic alcohol	3.96 mmol/L
	Peroxidase	≥1000 U/L
	4-aminoantipyrine	0.5 mmol/L
	Cholesterol oxidase	≥100 U/L
	Cholesterol esterase	≥100 U/L
Standard:	Cholesterol	200 mg/dl

Procedure:

	Blank	Sample/Standard
Sample/standard	-	10 µl
Distilled water	10 µl	-
Reagent 1	1000 µl	1000 µl

Mixed, incubated for 5 minutes at 37ºC. Absorbance was read against the blank at 546 nm within 60 minutes.

Calculation:

$$\text{Cholesterol in mg/dl} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

3.7.1.4. High Density Lipoprotein (HDL)

HDL was estimated by Heparin – Manganese precipitation procedure, Warnick, G.R and Albers, J.J (1978)

Reagents:

Heparin Sodium (40,000 USP unit/ml or 280 mg/ml)

Manganese chloride (1.06 M)

The reagent was prepared by mixing 0.6 ml of Heparin Sodium (280 mg/ml) with 10 ml of 1.06 M Manganese chloride.

Procedure:

1. To 100 µl of serum 10 µl of Heparin Manganese chloride reagent was added and mixed gently. After standing at room temperature for 10 min the mixture was centrifuged at 1500 x g for 30 min.
2. 10 µl of the clear supernatant was used for the estimation of HDL by Cholesterol oxidase phenol amino antipyrine (CHOD-PAP) method using Ecoline® kit (M/s E. Merck India Limited, Mumbai).

3.7.1.5. Low Density Lipoprotein (LDL)

LDL cholesterol was estimated by the conventional Friedewald Equation, (Warnick *et al.,* 1990)

LDL = Total Cholesterol – (HDL + VLDL)

3.7.1.6. Very Low Density Lipoprotein (VLDL)

VLDL cholesterol was estimated by the conventional Friedewald Equation, (Warnick *et al.,* 1990)

$$VLDL = \frac{\text{Triglycerides}}{5}$$

3.7.2. Analysis of liver and heart tissue

3.7.2.1. Estimation of lipid profile in liver and heart

Homogenates of liver and heart were prepared in a ratio of 1g of wet tissue to 9 ml of Isopropanol using a glass homogenizer. The tissue homogenate after storing at 4^{o}C for 2 days was centrifuged at 1000 x g for 10 minutes and the supernatant was used for the estimation of Triacylglycerides and Total cholesterol using commercially available kits.

3.7.2.2. Estimation of lipid peroxides (LP)

Level of lipid peroxides in tissue homogenate was determined by the method of Ohkawa *et al*. (1979).

Principle:

Thiobarbituric acid reacts with lipid peroxides and malondialdehyde to form a red coloured pigment that can be determined by colorimetry. TMP was used as a standard since it can be converted to malondialdehyde quantitatively by reacting with TBA.

Reagents:

TBA and TMP were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Sodium dodecyl sulphate (SDS) was procured from Sigma- Aldrich India, Bangalore; and all the other chemicals were purchased from Merck India Limited, Mumbai.

8.1% SDS

20% acetic acid solution, pH adjusted to 3.5 with NaOH

0.8% aqueous solution of TBA

1.15% KCl

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and heart were prepared in a ratio of 1g of wet tissue to 9 ml of 1.15% KCl solution (10% w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 50 x g for 5 minutes and the supernatant was used for the estimation of lipid peroxides.

To 100 µl of the supernatant, added 200 µl of 8.1% SDS, 1.5 ml 20% acetic acid solution (pH 3.5) and 1.5 ml of 0.8% aqueous solution of TBA. The mixture was made up to 4 ml with distilled water, and heated in a water bath at 95^{o}C for 60 minutes. After cooling under tap water, 1 ml of distilled water and

5ml of n-butanol were added and shaken vigorously. After centrifugation at 40 x g for 10 minutes, absorbance of the organic layer was taken at 532 nm.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 0.5 *nM* to 5 *nM* of TMP in deionised double distilled water by following the above procedure. A graph was plotted between optical density and concentration of the standards. The level of lipid peroxides were read directly from the standard curve, and expressed as nmol of malondialdehyde/g wet tissue.

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3.7.2.3. Estimation of reduced glutathione (GSH)

Level of reduced glutathione in serum and tissue homogenate was estimated by the method of Moron *et al*. (1979).

Principle:

Reduced glutathione is measured by its reaction with 5-5' dithiobis 2-nitro benzoic acid (DTNB) to give a yellow coloured complex with an absorption maxima at 412 nm.

Reagents:

Disodium hydrogen phosphate, monosodium dihydrogen phosphate and DTNB were purchased from Himedia Laboratories Pvt. Ltd, Mumbai. Trichloro acetic acid (TCA) was procured from Qualigens Fine Chemicals, Glaxo Smith Kline Pharmaceuticals Ltd, Mumbai. Mono and disodium hydrogen phosphates were used for the preparation of phosphate buffer.

0.2 *M* phosphate buffer, pH 8

25% TCA

5% TCA

0.6 mmol DTNB

Procedure:

Preparation of tissue homogenate:

Homogenates of liver and heart were prepared in a ratio of 1g of wet tissue to 9ml of 0.2 M phosphate buffer, pH 8 (10%w/v) using a glass homogenizer. The tissue homogenate was centrifuged at 50 x g for 5 minutes and the supernatant was used for the estimation of reduced glutathione.

Added 125 µl of 25% TCA to 500 µl of supernatant/ serum for the precipitation of proteins. The tubes were cooled on ice for 5 minutes and the mixture was further diluted with 575 µl of 5% TCA. Centrifuged the tubes for 5 minutes and 300 µl of resulting supernatant was taken for the estimation of reduced glutathione. The volume of aliquot was made up to 1 ml with 0.2 M phosphate buffer (pH, 8). Added 2ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. The level of reduced glutathione was measured from the standard curve.

Preparation of standard curve:

Standard curve was prepared using concentrations varying from 1 µg to 10µg of reduced glutathione which was dissolved in 5% TCA. The volume of standard solution was made up to 1 ml with 0.2 *M* phosphate buffer (pH, 8). Added 2ml of freshly prepared 0.6 mmol DTNB to the tubes and the intensity of yellow colour formed was read at 412 nm. A graph was plotted between optical density and concentration of the standards. Knowing the optical density of the unknown samples, the corresponding concentration of GSH was read directly

from the calibration curve and expressed as µg/ml in the case of serum and µg/g wet tissue in the case of liver and heart.

3.7.2.4 Estimation of liver glycogen

Liver glycogen was estimated using Anthrone reagent (Carroll *et al.,* 1956).

Principle:

Sulphuric acid medium of anthrone reagent causes dehydration of sugar to a furfural derivative, which presumably condenses with anthrone to form a blue colored compound, which was measured in a spectrophotometer at 620 nm.

Reagents:

1. Anthrone reagent (0.05%): The reagent was prepared by dissolving 0.05 g of anthrone in 100 ml of 72 % sulphuric acid. The reagent was prepared fresh whenever required.
2. KOH solution (30%): Dissolved 300 g of reagent grade potassium hydroxide pellets in distilled water in a beaker, cooled and transferred quantitatively into one litre volumetric flask and diluted to one litre with distilled water.
3. 95 per cent ethanol.
4. Glucose standard: (a) Stock solution: Dissolved 100 mg of dry, highest purity glucose in 100 ml of distilled water (b) Working standard: Diluted 0.1 ml of the stock solution to 1 ml with distilled water. 0.5 ml of this solution, containing 0.05 mg of glucose was used as standard.

Procedure:

Approximately 0.1 g of liver tissue was taken in a test tube containing 1 ml of boiled 30% KOH solution. The tissue was digested by heating the tube for 15 minutes in a boiling water bath. The sample was then cooled and diluted to 10 ml with distilled water in a test tube. After thorough mixing in a vortex, 1 ml of the solution was pipetted into a second tube and again diluted to 10 ml with distilled water. From this dilution 0.5 ml was taken in a centrifuge tube and added 0.6 ml (1.2 volume) of 95% ethanol to precipitate glycogen, which was kept overnight. Centrifuged the tubes at 30 x g for 15 minutes and discarded the supernatant by keeping the tubes inverted. Dissolved the pellet completely in 0.5 ml distilled water by mixing in a vortex. This was taken as the unknown sample.

Standard: 0.5 ml of glucose working standard

Blank: 0.5 ml of distilled water

The sample, standard and blank were kept in a cold water bath and added 2.5 ml of anthrone reagent to each of the three tubes from a fast flowing burette. Mixed the reactants thoroughly in a vortex. After cooling, covered the mouth of test tubes with glass stoppers and heated for 15 minutes in boiling water bath. Then immediately cooled by placing them in cold water bath. The optical densities were taken against the blank at 620 nm in a spectrophotometer.

Calculation - The calculation of glycogen is as follows:

$$\frac{DU}{DS} \times 0.05 \times \frac{\text{volume of extract}}{\text{gram of tissue}} \times 100 \times 0.9 \times 10 = \text{mg of glycogen per 100 gm tissue}$$

Where, DU = optical density of the unknown

DS = optical density of the standard

0.05 = mg of glucose in 0.5 ml of standard solution

0.9 = factor for converting glucose value to glycogen value.

10 = Dilution factor

100 = in 100 gram of tissue

Volume of extract = 10 ml

Gram of tissue = 0.1 g

3.7.3. Histopathological examination of liver and heart

Representative samples of liver and heart obtained from the dissected animals were fixed in 10% neutral buffered formalin. They were processed and paraffin embedded as described by Sheehan and Hrapchak, (1980). The sections were stained with haematoxyline and eosin as per the technique followed by Bancroft and Cook, (1984). The sections were examined in detail under light microscope.

3.8. STATISTICAL ANALYSIS OF THE DATA

Data obtained were analyzed by the following statistical tools (Snedecor and Cochran, 1994) to determine the level of significance. The value of $P<0.05$ was considered statistically significant.

1. Tissue parameters was compared by analysis of variance (ANOVA) followed by Duncan's multiple range test.
2. Body weight and serum metabolites for different groups was analyzed by analysis of co-variance (ANCOVA).

4. RESULTS

In the present study, effect of various preparations of coconut oil and fish oil was evaluated on lipid profile and antioxidant status in rats. The results were analyzed and presented in tables and figures.

4.1. BODY WEIGHT

Body weight of different groups is presented in Table 1 and Fig.1. On day 45, no significant difference was observed in the body weight of G4 (199.48 ± 5.71 g), while all other oil administered groups showed significant ($P < 0.05$) increase when compared with G1 (205.20 ± 5.74 g). On day 90, G2 (251.06 ± 3.88 g) and G3 (232.31 ± 3.84 g) showed a body weight similar to that of G1 (241.56 ± 3.86 g) whereas, that of G4 was significantly ($P < 0.05$) lower and G5 was significantly ($P < 0.05$) higher than G1.

Comparison between groups, after 90 days of oil administration, showed a significantly ($P < 0.05$) higher body weight in G2 than G3. Highest body weight was exhibited by G5 and lowest by G4 and both were significantly ($P < 0.05$) different from that of the other two groups.

4.2. SERUM BIOCHEMISTRY

4.2.1. Total lipids (TL) and Triacylglycerol (TAG)

Serum TL and TAG concentrations of different groups are presented in Table 2 and Fig. 2 and 3 respectively. Compared with G1, all the groups administered with oil showed a significant ($P < 0.05$) increase in the level of TL on day 45. Among the oil administered groups, G2 (647.38 ± 8.33 mg/dl) and G3 (620.64 ± 8.27 mg/dl) showed a value nearly double than that of G1 (333.16 ± 8.51 mg/dl).

Table 1. Effect of administration of different oils on body weight (g)

(Values are Mean ± SE of 6 animals)

Groups	Days		
	0	45	90
G1	$202.17^{a} \pm 2.95$	$205.20^{a} \pm 5.74$	$241.56^{a,b} \pm 3.86$
G2	$201.83^{a} \pm 1.64$	$260.68^{b} \pm 5.76$	$251.06^{a} \pm 3.88$
G3	$202.67^{a} \pm 1.67$	$224.15^{c} \pm 5.71$	$232.31^{b} \pm 3.84$
G4	$202.67^{a} \pm 1.71$	$199.48^{a} \pm 5.71$	$213.81^{c} \pm 3.84$
G5	$207.50^{a} \pm 1.59$	$249.16^{b} \pm 6.17$	$287.09^{d} \pm 4.15$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$).

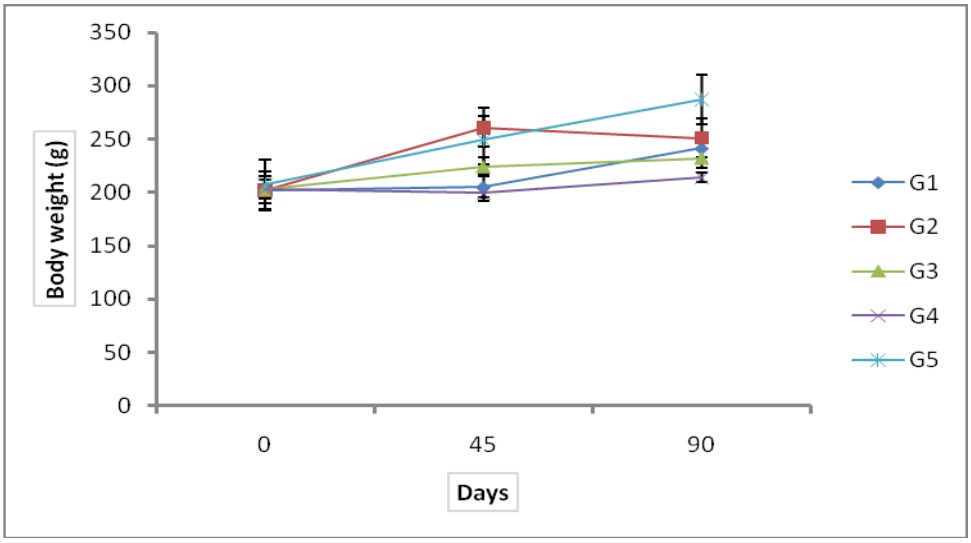

Fig. 1. Effect of administration of different oils on body weight (g)

G1 -NC, G2 - CO, G3 - SCO, G4 - VCO, G5 - FO

Table 2. Effect of administration of different oils on the levels of serum total lipids and triacyglycerol (mg/dl)

(Values are Mean ± SE of 6 animals)

Groups	Total Lipids (TL)			Triacylglycerol (TAG)		
	Days			Days		
	0	45	90	0	45	90
G1	337.68^{a} ± 8.72	333.16^{a} ± 8.51	334.33^{a} ± 5.94	41.33^{a} ± 1.98	43.22^{a} ± 3.89	44.35^{a} ± 2.37
G2	356.88^{a} ± 7.83	647.38^{b} ± 8.33	353.72^{a} ± 5.81	49.00^{a} ± 5.37	60.12^{b}± 3.91	68.81^{b}± 2.38
G3	345.22^{a} ± 7.87	620.64^{c} ± 8.27	402.24^{c} ± 5.77	46.17^{a} ± 2.01	71.65^{c} ± 3.82	74.35^{b} ± 2.33
G4	356.82^{a} ±10.68	366.37^{d} ± 8.33	323.15^{a} ± 5.81	45.83^{a} ± 3.39	54.24^{a,b}± 3.82	43.15^{a} ± 2.32
G5	352.12^{a} ±10.75	363.67^{d} ± 8.24	275.62^{d} ± 5.75	43.00^{a} ± 3.59	77.10^{c} ± 3.84	53.52^{c} ± 2.34

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$).

G1 -NC, G2 - CO, G3 - SCO, G4 - VCO, G5 - FO

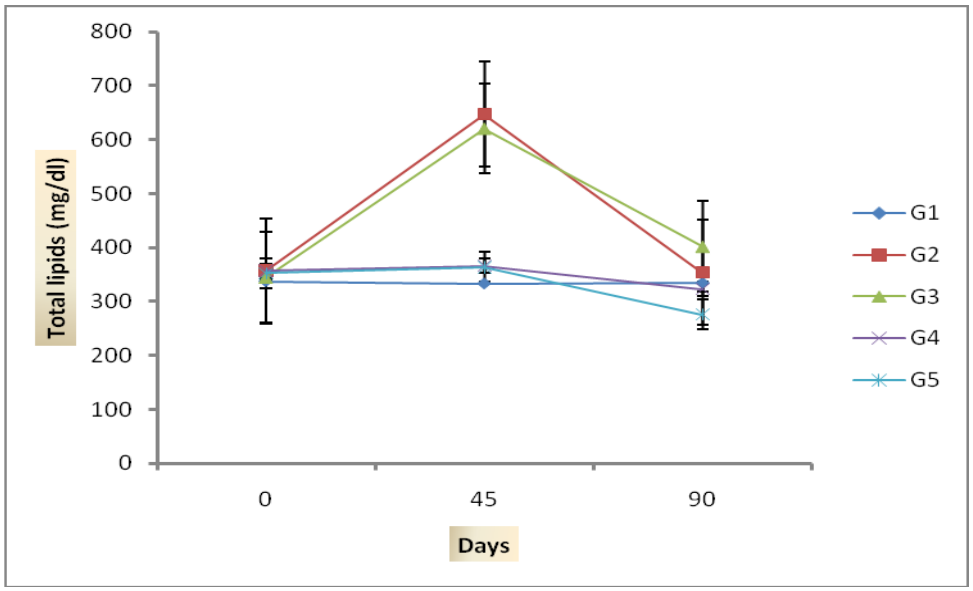

Fig. 2. Effect of administration of different oils on the levels of serum total lipids (mg/dl)

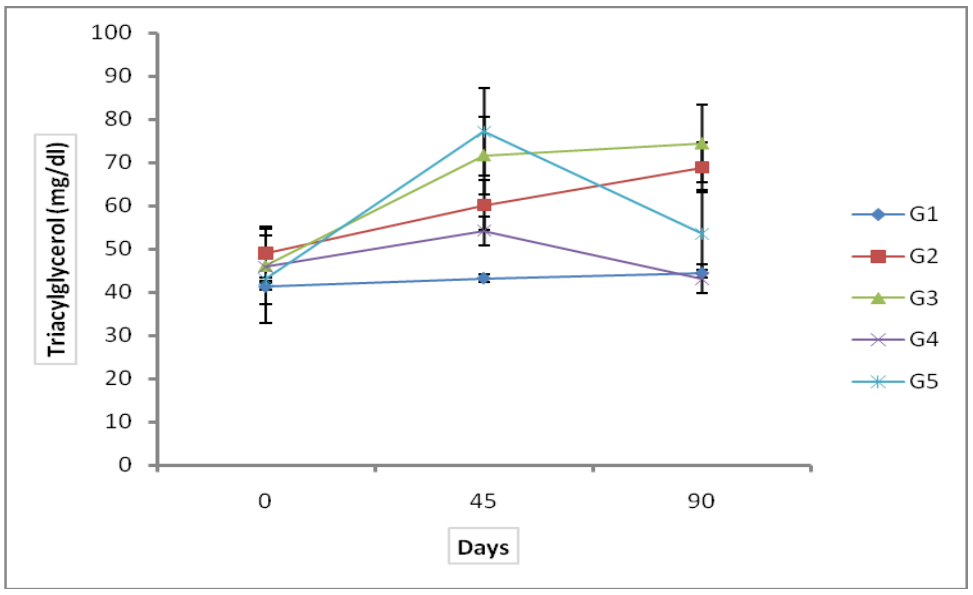

Fig. 3. Effect of administration of different oils on the levels of serum triacyglycerol (mg/dl)

G1 -NC, G2 - CO, G3 - SCO, G4 - VCO, G5 - FO

Administration of oils for 90 days reduced the level of TL considerably in G2 (353.72 ± 5.81 mg/dl), which was similar to that of G1. Although, there was a substantial reduction in the level of TL in G3 (402.24 ± 5.77 mg/dl) compared to day 45, it was significantly ($P < 0.05$) higher than that of G1 (334.33 ± 5.94 mg/dl). On day 90 the level of TL in G4 (323.15 ± 5.81 mg/dl) was similar to that of G1, while a significant ($P < 0.05$) reduction was observed in G5 (275.62 ± 5.75 mg/dl) when compared with G1.

Except G4, all other oil administered groups showed a significantly ($P < 0.05$) higher level of serum TAG on day 45 compared with G1 (43.22 ± 3.89 mg/dl) but G4 had a numerical increase in TAG than G1. On day 90, the level of TAG in G4 (43.15 ± 2.32 mg/dl) was similar to that of G1 (44.35 ± 2.37 mg/dl). In G2 (68.81± 2.38 mg/dl) and G3 (74.35 ± 2.33 mg/dl) TAG remained at a significantly ($P < 0.05$) higher level, while in G5 (53.52 ± 2.34 mg/dl) it decreased considerably compared to 45 day, though, it was significantly ($P < 0.05$) higher when compared with G1.

Comparison between oil administered groups after 3 months revealed that TL was highest in G3 and lowest in G5 and similar values were observed in G2 and G4. Between oil administered groups, significantly higher TAG level was observed in G2 and G3 and no significant variation was noted between G2 and G3. Between G4 and G5, G5 showed a significantly ($P < 0.05$) higher TAG level.

4.2.2. Total cholesterol (TC) and HDL

Serum TC and HDL levels of different groups are presented in Table 3 and Fig.4 and 5 respectively. On day 45, significant ($P<0.05$) increase was observed in the level of serum TC for all the oil administered groups except G5 (41.52 ± 2.35 mg/dl), which showed a value similar to that of G1 (47.74 ± 2.45 mg/dl). Administration of oils for 90 days showed similar values in G2 (47.86 ± 1.75 mg/dl), G4 (47.46 ± 1.76 mg/dl) and G5 (43.49 ± 1.74 mg/dl) to that of G1, while

Table 3. Effect of administration of different oils on the levels of serum total cholesterol and HDL cholesterol (mg/dl)

(Values are Mean ± SE of 6 animals)

Groups	Total cholesterol (TC)			HDL		
	Days			Days		
	0	45	90	0	45	90
G1	$47.33^{a} \pm 1.45$	$47.74^{a} \pm 2.45$	$48.08^{a} \pm 1.82$	$19.17^{a} \pm 0.54$	$19.09^{a} \pm 1.19$	$19.65^{a} \pm 1.07$
G2	$45.67^{a} \pm 2.26$	$59.70^{b} \pm 2.36$	$47.86^{a} \pm 1.75$	$18.17^{a} \pm 1.05$	$31.04^{b} \pm 1.19$	$20.34^{a} \pm 1.07$
G3	$43.17^{a} \pm 1.14$	$60.16^{b} \pm 2.40$	$68.44^{b} \pm 1.78$	$17.50^{a} \pm 0.81$	$26.90^{c} \pm 1.19$	$28.53^{b} \pm 1.08$
G4	$43.67^{a} \pm 1.23$	$56.06^{b} \pm 2.38$	$47.46^{a} \pm 1.76$	$18.67^{a} \pm 0.88$	$27.15^{c} \pm 1.19$	$27.49^{b} \pm 1.07$
G5	$44.83^{a} \pm 1.70$	$41.52^{a} \pm 2.35$	$43.49^{a} \pm 1.74$	$18.67^{a} \pm 2.09$	$17.48^{a} \pm 1.19$	$20.99^{a} \pm 1.07$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 - FO

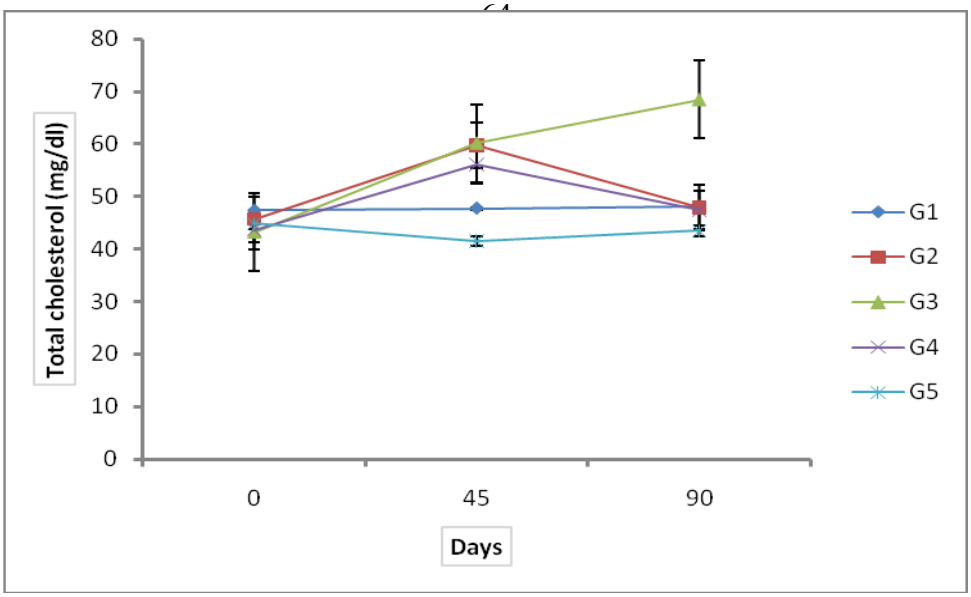

Fig. 4. Effect of administration of different oils on the levels of serum total cholesterol (mg/dl)

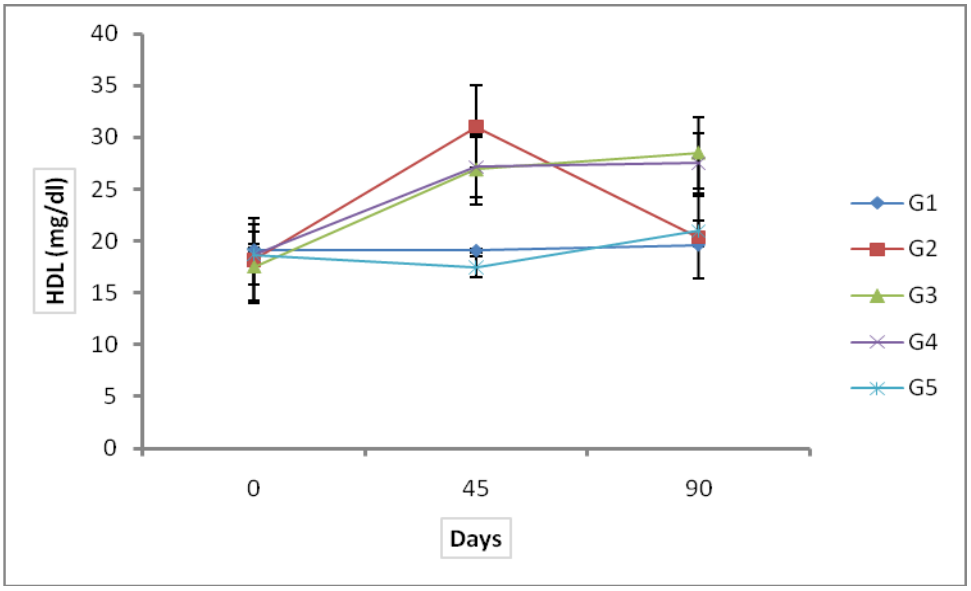

Fig. 5. Effect of administration of different oils on the levels of serum HDL cholesterol (mg/dl)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

in G3 (68.44 ± 1.78 mg/dl) a significant ($P < 0.05$) increase was noticed.

On day 45, a significant ($P<0.05$) increase was observed in the serum HDL cholesterol level of all the oil administered groups except G5 (17.48 ± 1.19 mg/dl), which showed a value similar to that of G1 (19.09 ± 1.19 mg/dl). Administration of oil for 90 days, did not show any significant variation in the level of serum HDL in G2 (20.34 ± 1.07 mg/dl) and G5 (20.99 ± 1.07 mg/dl) whereas, G3 (28.53 ± 1.08 mg/dl) and G4 (27.49 ± 1.07 mg/dl) showed significantly ($P < 0.05$) higher levels, when compared with that of G1 (19.65 ± 1.07 mg/dl).

Comparison between oil administered groups on day 90, revealed a significantly ($P < 0.05$) higher TC level in G3 and all other groups showed similar values while, HDL level was significantly ($P < 0.05$) higher in G3 and G4 compared to the other two groups. No significant variation was observed between G3 and G4 and between G2 and G5.

4.2.3. LDL and VLDL

Serum LDL and VLDL levels of different groups are presented in Table 4 and Fig. 6 and 7 respectively. On day 45, serum LDL decreased significantly ($P < 0.05$) in G2 (13.58 ± 1.34 mg/dl) and G5 (10.98 ± 1.34 mg/dl) whereas, in G3 (21.13 ± 1.34 mg/dl) it increased though, not significantly when compared with G1 (15.84 ± 1.38 mg/dl). The level of LDL in G4 (14.74 ± 1.34 mg/dl) did not differ significantly from that of G1. Administration of oils for 90 days significantly ($P < 0.05$) reduced the level of LDL in G2 (11.45 ± 1.37 mg/dl), G4 (7.64 ± 0.37 mg/dl) and G5 (10.94 ± 1.36 mg/dl) while a significant ($P < 0.05$) increase was observed in G3 (25.05 ± 1.3 mg/dl) when compared with G1 (15.50 ± 1.41 mg/dl).

Table. 4. Effect of administration of different oils on serum LDL and VLDL (mg/dl)

(Values are Mean ± SE of 6 animals)

Groups	LDL			VLDL		
	Days			Days		
	0	45	90	0	45	90
G1	$15.37^{a} \pm 0.75$	$15.84^{a,c} \pm 1.38$	$15.50^{a} \pm 1.41$	$12.73^{a} \pm 0.56$	$12.93^{a} \pm 0.75$	$12.66^{a} \pm 0.53$
G2	$14.93^{a} \pm 1.68$	$13.58^{b} \pm 1.34$	$11.45^{b} \pm 1.37$	$12.67^{a} \pm 0.71$	$14.77^{b} \pm 0.83$	$15.29^{b} \pm 0.58$
G3	$15.33^{a} \pm 1.12$	$21.13^{c} \pm 1.34$	$25.05^{c} \pm 1.3$	$12.30^{a} \pm 0.29$	$12.85^{a} \pm 0.78$	$15.53^{b} \pm 0.54$
G4	$15.10^{a} \pm 1.84$	$14.74^{a,b} \pm 1.34$	$7.64^{b} \pm .37$	$11.67^{a} \pm 0.41$	$13.57^{b} \pm 0.75$	$12.24^{a} \pm 0.53$
G5	$15.50^{a} \pm 0.85$	$10.98^{b} \pm 1.34$	$10.94^{b} \pm 1.36$	$11.63^{a} \pm 0.81$	$12.95^{a} \pm 0.76$	$11.02^{a} \pm 0.53$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

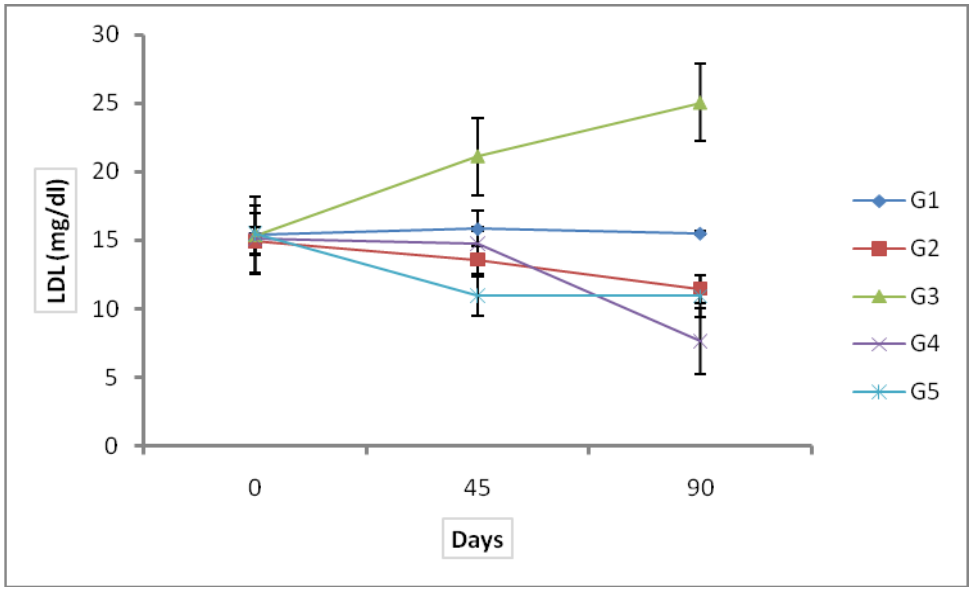

Fig. 6. Effect of administration of different oils on serum LDL (mg/dl)

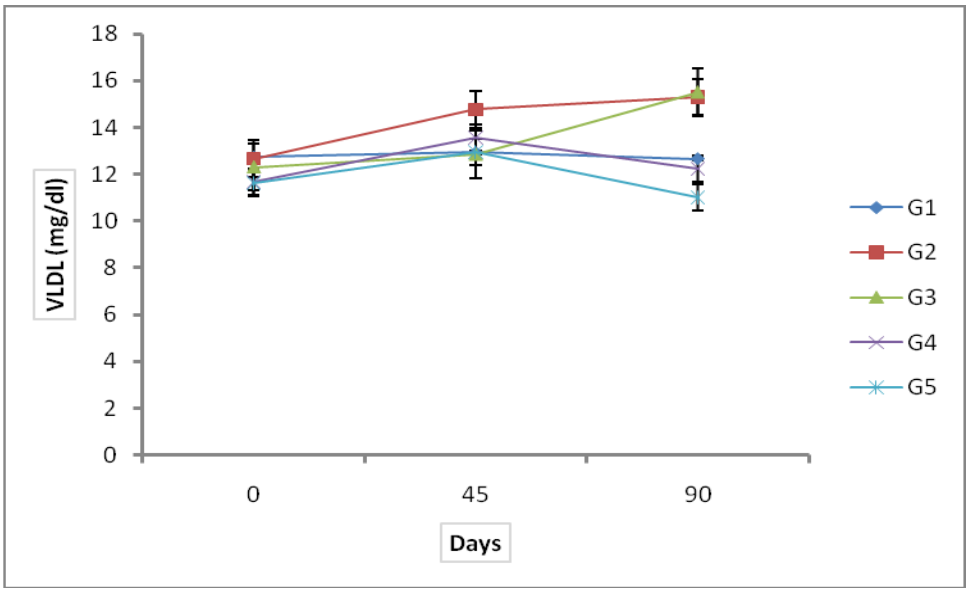

Fig. 7. Effect of administration of different oils on serum VLDL (mg/dl)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

Level of serum VLDL increased significantly ($P < 0.05$) in G2 (14.77 ± 0.83 mg/dl) and G4 (13.57 ± 0.75 mg/dl) on day 45 while no significant difference was observed in G3 (12.85 ± 0.78 mg/dl) and G5 (12.95 ± 0.76 mg/dl) when compared with G1 (12.93 ± 0.75 mg/dl).Administration of oil for 90 days also showed a significantly ($P < 0.05$) higher level of serum VLDL in G2 (15.29 ± 0.58 mg/dl) and in G3 (15.53 ± 0.54 mg/dl) when compared with G1. In G4 (12.24 ± 0.53 mg/dl) and G5 (11.02 ± 0.53 mg/dl) it did not differ significantly from that of G1 (12.66 ± 0.53 mg/dl).

Comparison between groups after 90 days of oil administration revealed a significantly ($P < 0.05$) higher level of LDL in G3 and no variation was observed between the other groups. However, a lowest serum LDL level was observed in G4. Between oil administered groups, VLDL level was significantly higher in G2 and G3 compared to the other two groups. No significant difference was observed between G2 and G3 and between G4 and G5.

4.2.4. HDL/LDL ratio

Serum HDL/LDL ratio of different groups is presented in Table 5 and Fig. 8. On day 45 significant ($P<0.05$) increase was observed in serum HDL/ LDL ratio of all oil administered groups except G3 when compared with G1. On day 90, G4 (3.85 ± 0.41) showed the maximum value of HDL/LDL ratio followed by G2 and G5 compared to G1 (1.11 ± 0.08). In G3, slight increase was observed in the ratio on day 45, which decreased to the basal level (day 0) by day 90 (1.17 ± 0.09). Comparison between oil administered groups on day 90 showed a maximum ratio of HDL/LDL in G4 followed by G2, G5 and lowest in G3.

4.3. WEIGHT OF LIVER AND HEART

Weight of liver and heart of different groups is presented in Table 6 and Fig. 9. Weight of liver in G3 (7.23 ± 0.17 g) decreased significantly ($P < 0.05$)

Table 5. Effect of administration of different oils on HDL/LDL ratio

Groups	Days		
	0	45	90
G1	$1.15^{a} \pm 0.06$	$1.12^{a} \pm 0.03$	$1.11^{a} \pm 0.08$
G2	$1.29^{a} \pm 0.17$	$2.45^{b} \pm 0.32$	$2.09^{b} \pm 0.31$
G3	$1.17^{a} \pm 0.11$	$1.25^{a} \pm 0.11$	$1.17^{a} \pm 0.09$
G4	$1.33^{a} \pm 0.15$	$2.04^{b,c} \pm 0.34$	$3.85^{c} \pm 0.41$
G5	$1.23^{a} \pm 0.15$	$1.60^{c} \pm 0.17$	$2.04^{b} \pm 0.22$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

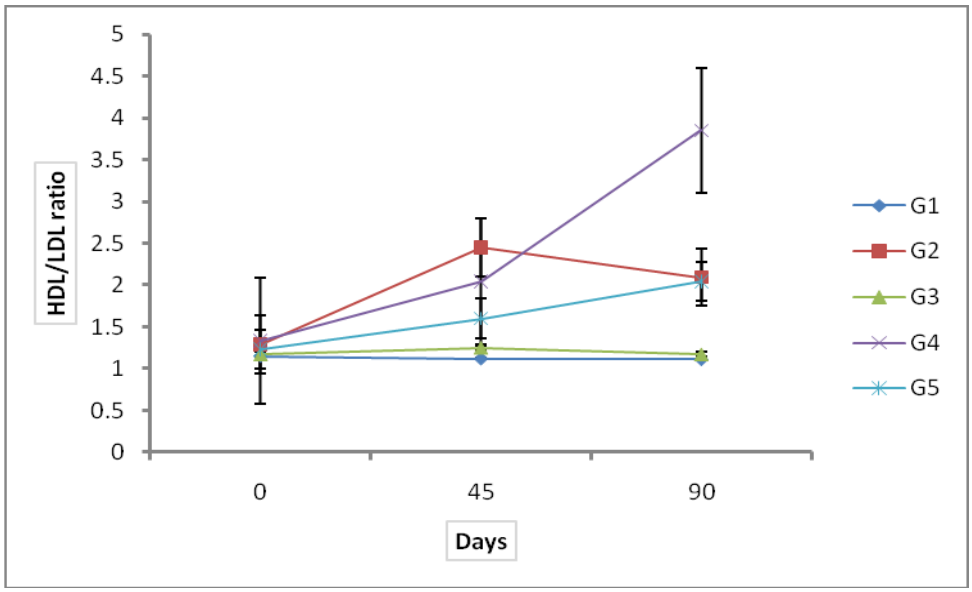

Fig. 8. Effect of administration of different oils on HDL/LDL ratio

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

Table. 6. Effect of administration of different oils on weight of liver and heart (g)

(Values are Mean ± SE of 6 animals)

Groups	Liver	Heart
G1	$8.48^{a} \pm 0.42$	$0.66^{a} \pm 0.03$
G2	$10.34^{c} \pm 0.18$	$0.79^{b} \pm 0.02$
G3	$7.23^{b} \pm 0.17$	$0.67^{a} \pm 0.01$
G4	$8.45^{a} \pm 0.38$	$0.70^{a} \pm 0.02$
G5	$10.61^{c} \pm 0.39$	$0.83^{b} \pm 0.01$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

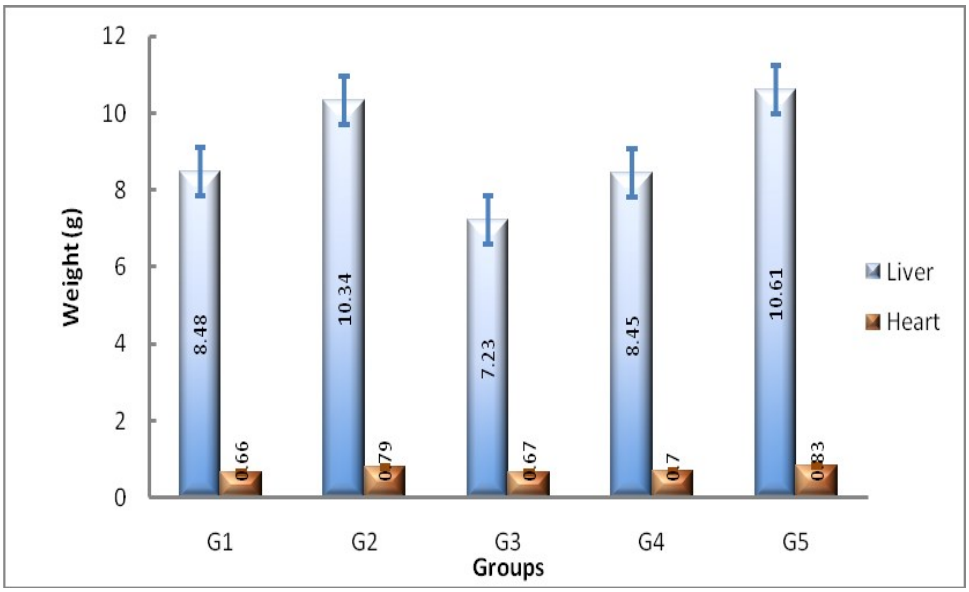

Fig. 9. Effect of administration of different oils on weight of liver and heart (g)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

while that of heart (0.67 ± 0.01 g) did not show any significant variation from that of G1 (Liver - 8.48 ± 0.42 g, Heart - 0.66 ± 0.03 g). Both liver and heart showed a significant ($P < 0.05$) increase in weight in G2 (Liver - 10.34 ±0.18 g, Heart - 0.79 ± 0.02g) and G5 (Liver - 10.61 ± 0.39 g, Heart - 0.83 ± 0.01 g) whereas, in G4 (Liver - 8.45 ± 0.38g, Heart - 0.70 ± 0.02 g) weight of both the organs was homogenous to that of G1.

Comparison between different oil administered groups showed significantly ($P < 0.05$) higher weight of both the organs in G2 and G5, which did not differ significantly between these groups. Weight of liver was significantly ($P < 0.05$) lower in G3 than G4 while that of heart did not differ significantly between these two groups.

4.4. BIOCHEMISTRY OF LIVER AND HEART

4.4.1. Total cholesterol (TC) and Triacylglycerol (TAG)

Levels of TC and TAGs in liver and heart of different groups are presented in Table 7 and Fig. 10 and 11 respectively. Significantly ($P < 0.05$) higher levels of TC was observed in tissues of heart of G2 (Heart - 1.83 ± 0.11 mg/g) and liver and heart of G3 (Liver - 2.28 ± 0.11 mg/g, Heart - 1.80 ± 0.09 mg/g), but the level of TC in liver did not differ significantly in G2 (Liver - 2.16 ± 0.08 mg/g) when compared with NC. On the contrary, TC decreased significantly ($P < 0.05$) in both liver and heart of G4 (Liver - 1.35 ± 0.07 mg/g, Heart - 0.89 ± 0.03 mg/g) and liver of G5 (Liver - 1.70 ± 0.04 mg/g) while, the level did not differ significantly in heart tissues of G5 (Heart - 1.10 ± 0.06 mg/g) when compared with G1 (Liver - 2.01 ± 0.11 mg/g, Heart - 1.30 ± 0.06 mg/g).

A significantly ($P < 0.05$) higher level of TAG was observed in liver and heart of G2 (Liver – 41.61 ± 2.61 mg/g, Heart - 6.44 ± 0.59 mg/g) and liver TAG level was about 10 times more when compared with G1 (Liver - 4.41 ± 0.35 mg/g, Heart - 1.91 ± 0.14 mg/g). In all other oil administered groups the level of TAG in

Table 7. Effect of administration of different oils on total cholesterol and triacylglycerol levels in liver and heart (mg/g)

(Values are Mean ± SE of 6 animals)

Groups	Total cholesterol (TC)		Triacylglycerol (TAG)	
	Liver	Heart	Liver	Heart
G1	$2.01^{a} \pm 0.11$	$1.30^{a} \pm 0.06$	$4.41^{a} \pm 0.35$	$1.91^{a} \pm 0.14$
G2	$2.16^{a,b} \pm 0.08$	$1.83^{b} \pm 0.11$	$41.61^{b} \pm 2.61$	$6.44^{b} \pm 0.59$
G3	$2.28^{b} \pm 0.11$	$1.80^{b} \pm 0.09$	$5.35^{a} \pm 0.20$	$1.90^{a} \pm 0.09$
G4	$1.35^{c} \pm 0.07$	$0.89^{c} \pm 0.03$	$4.49^{a} \pm 0.19$	$3.06^{c} \pm 0.15$
G5	$1.70^{d} \pm 0.04$	$1.10^{a,c} \pm 0.06$	$5.88^{a} \pm 0.41$	$1.66^{a} \pm 0.19$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$).

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

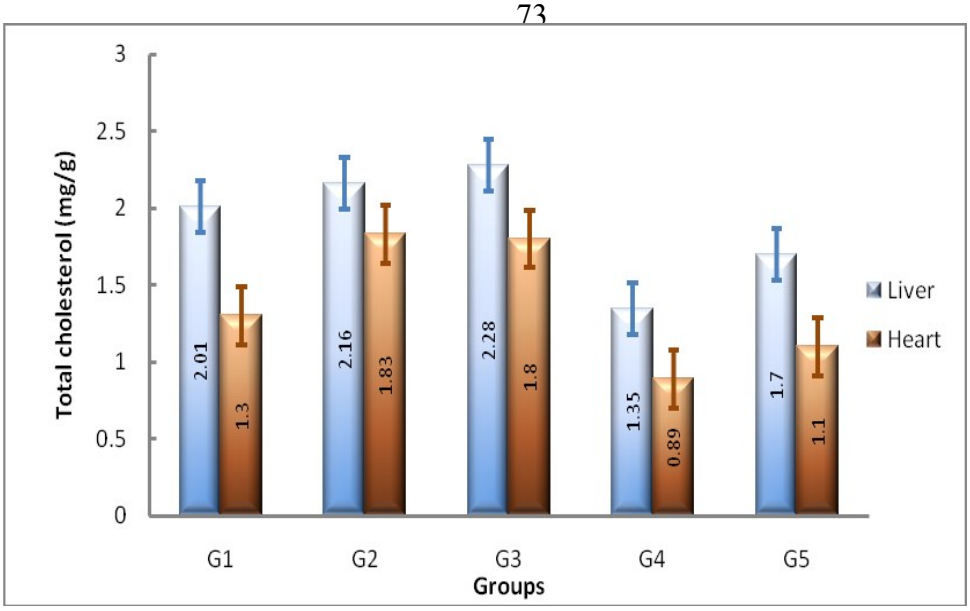

Fig. 10. Effect of administration of different oils on total cholesterol levels in liver and heart (mg/g)

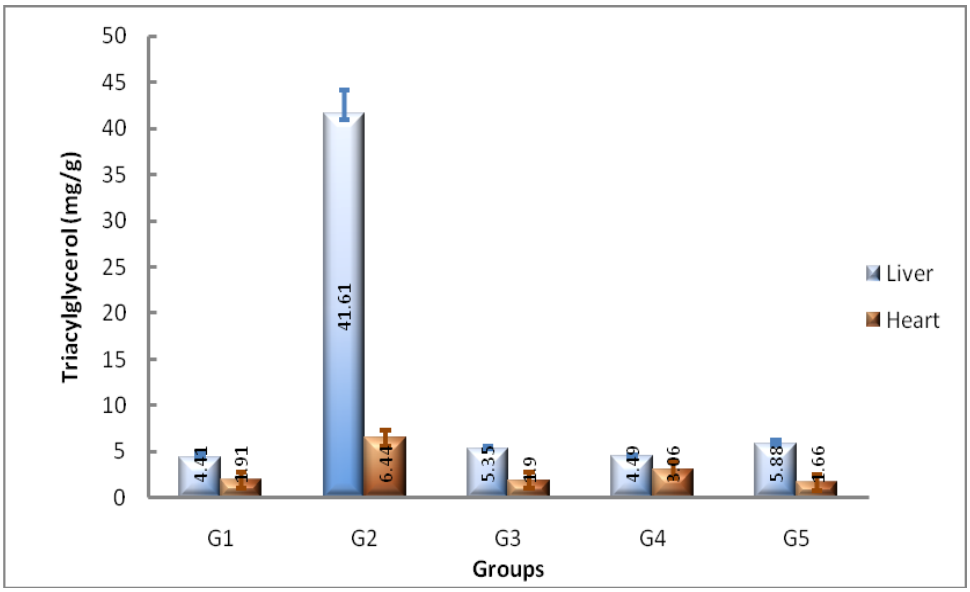

Fig. 11. Effect of administration of different oils on triacylglycerol levels in liver and heart (mg/g)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

liver and heart did not differ significantly except for heart in G4 (3.06 ± 0.15 mg/g) in which it was significantly ($P < 0.05$) higher when compared with G1.

Comparison between oil administered groups did not exhibit any significant difference in the level of TC in liver and heart of G2 and G3 while, it was significantly lower than these two groups in both tissues of G4 and G5. Lowest level of TC was observed in G4. In both the tissues, highest TAG level was observed in G2, which was significantly ($P < 0.05$) different from all other groups. Except for a significantly ($P < 0.05$) higher TAG level in heart of G4, no significant variation was observed in the level of TAG in liver and heart of G3, G4 and G5

4.4.2. Lipid peroxides (LP) and reduced glutathione (GSH)

Level of LP and GSH in liver and heart of different groups are presented in Table 8 and Fig. 12 and 13 respectively. Significant ($P < 0.05$) increase in LP was observed in G2 (Liver - 464.38 ± 12.08 nM/g, Heart - 370.63 ± 20.08 nM/g) and G5 (Liver - 749.13 ± 40.49 nM/g, Heart - 435.63 ± 16.97 nM/g) whereas, in G3 and G4 its level did not differ significantly in both tissues compared with G1 (Liver - 375.00 ± 23.13 nM/g, Heart - 302.50 ± 11.76 nM/g).

Level of GSH in liver decreased significantly ($P < 0.05$) in G4 (Liver - 500.00 ± 20.00 μg/g) and did not show any significant variation in all other oil administered groups when compared with G1 (Liver - 850.00 ± 76.99 μg/g). However, in G2 the level of GSH in liver was higher though not significant when compared with G1, but significantly ($P < 0.05$) higher when compared with all other oil administered groups. In G4 (465.00 ± 15.00 μg/g), heart tissue also showed a significantly ($P < 0.05$) lower level of GSH whereas, in all other groups, a significantly higher level was observed compared with G1. When compared between oil administered groups, LP level was significantly ($P < 0.05$) higher in both the tissues of G2 and G5 compared to G3 and G4 and no significant variation was observed between G3 and G4. Between G2 and G5 significantly ($P < 0.05$) higher LP level was noted in G5 in both the tissues.

Table. 8. Effect of administration of different oils on tissue lipid peroxides (nM/g) and reduced glutathione (GSH) (µg/g)

(Values are Mean ± SE of 6 animals)

Groups	Lipid peroxides (LP)		Reduced glutathione (GSH)	
	Liver	Heart	Liver	Heart
G1	375.00^{a} ± 23.13	302.50^{a} ± 11.76	$850.00^{a,b}$ ± 76.99	695.00^{a} ± 23.83
G2	464.38^{b} ± 12.08	370.63^{b} ± 20.08	915.00^{b} ± 45.63	1125.00^{b} ± 36.59
G3	385.63^{a} ± 11.63	290.00^{a} ± 12.61	765.00^{a} ± 42.38	865.00^{c} ± 58.52
G4	367.50^{a} ± 13.56	275.63^{a} ± 9.13	500.00^{c} ± 20.00	465.00^{d} ± 15.00
G5	749.13^{c} ± 40.49	435.63^{c} ± 16.97	745.00^{a} ± 26.12	1160.00^{b} ± 52.37

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b, c and d) in a column differ significantly ($P<0.05$).

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

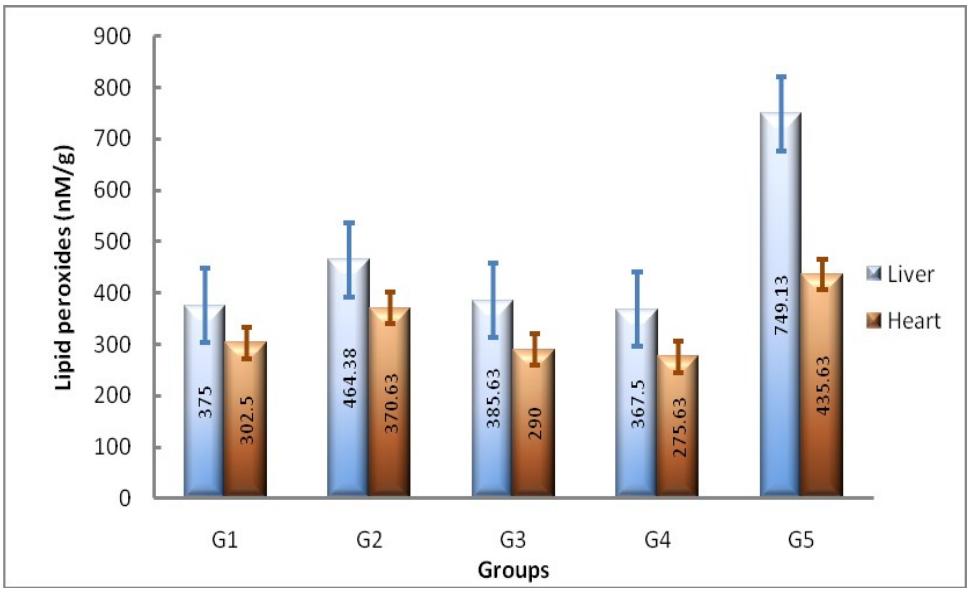

Fig. 12. Effect of administration of different oils on tissue lipid peroxides (nM/g)

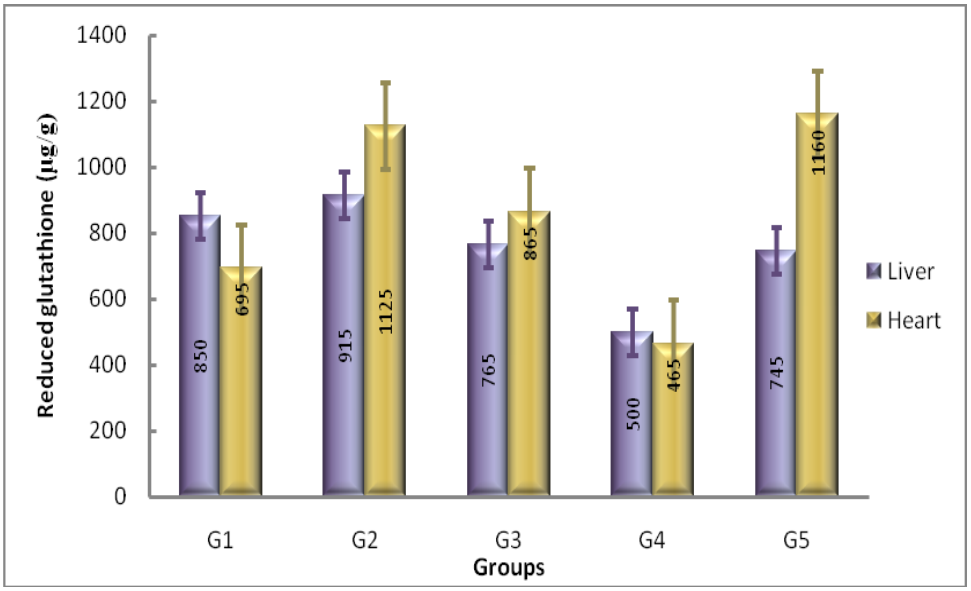

Fig. 13. Effect of administration of different oils on tissue reduced glutathione (GSH) (µg/g)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

Comparing the content of GSH in different oil administered groups, G2 showed a significantly ($P < 0.05$) higher level in both the tissues but the level in heart did not vary significantly between G2 and G5. Lowest GSH content was observed in G4 in both the tissues, which was significantly ($P < 0.05$) different from other groups. In G3 and G5 no significant variation was observed in liver GSH content, while in heart a significantly ($P < 0.05$) higher level was noted in G5 compared to G3.

4.4.3. Liver glycogen

Liver glycogen level of different groups is presented in Table 9 and Fig. 14. Level of liver glycogen decreased significantly ($P < 0.05$) in G2 (1.84 ± 0.12 g %), G3 (0.84 ± 0.02 g %) and G5 (0.63 ± 0.05 g %) while G4 (2.89 ± 0.11 g %) did not differ significantly when compared with G1 (2.83 ± 0.18 g %). G3 and G5 showed values, which were less than one third of G1.

Comparison between oil administered groups, revealed the highest level of Liver glycogen in G4, which was significantly ($P < 0.05$) different from other groups, followed by G2. Between G3 and G5, level of liver glycogen did not vary significantly but it was significantly ($P < 0.05$) lower when compared with the other two groups.

4.5. HISTOPATHOLOGY OF LIVER AND HEART

Representative samples of liver and heart of all groups were subjected to histopathological examination. Microscopic figures of liver and heart sections are presented in Fig. 15 (a, b, c, d and e) and 16 (a, b, c, d and e) respectively.

Microscopic examination of the liver (Fig. 15a) and heart (Fig. 16a) of G1 revealed normal histological architecture. In G2, changes could be seen in both liver (Fig. 15b) and heart (Fig. 16b). Diffuse necrosis, vacuolation of hepatocytes and central venous congestion were noticed in liver. Lesions in heart

Table. 9. Effect of administration of different oils on liver glycogen (g %)

(Values are Mean ± SE of 6 animals)

Groups	Liver Glycogen
G1	$2.83^{a} \pm 0.18$
G2	$1.84^{b} \pm 0.12$
G3	$0.84^{c} \pm 0.02$
G4	$2.89^{a} \pm 0.11$
G5	$0.63^{c} \pm 0.05$

Level of significance was determined column wise between groups.

Values not bearing a common superscript letter (a, b and c) in a column differ significantly ($P<0.05$).

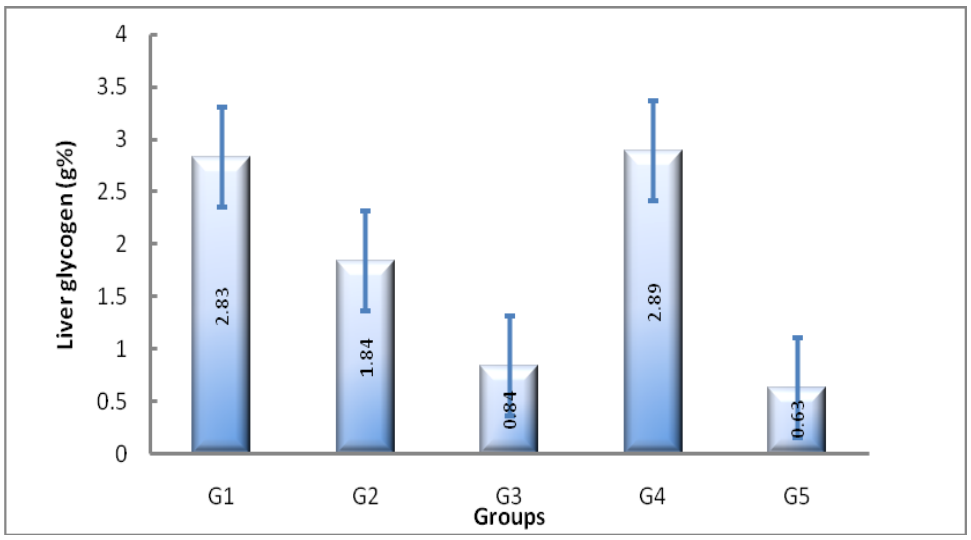

Fig. 14. Effect of administration of different oils on liver glycogen (g %)

G1 - NC, G2 - CO, G3 - SCO, G4 - VCO, G5 – FO

were characterized by congestion and moderate hyalinization of some of the heart fibres, amidst healthy cardiac fibres.

Liver sections (15c) in G3, showed dilatation of sinusoids and diffuse congestion with normal hepatocytes while cardiac muscle fibres (Fig. 16c) appeared normal. Examination of liver (Fig. 15d and 15e) and heart (Fig. 16d and 16e) of G4 and G5 revealed normal histology of both the organs except for a mild degeneration of hepatocytes in G4.

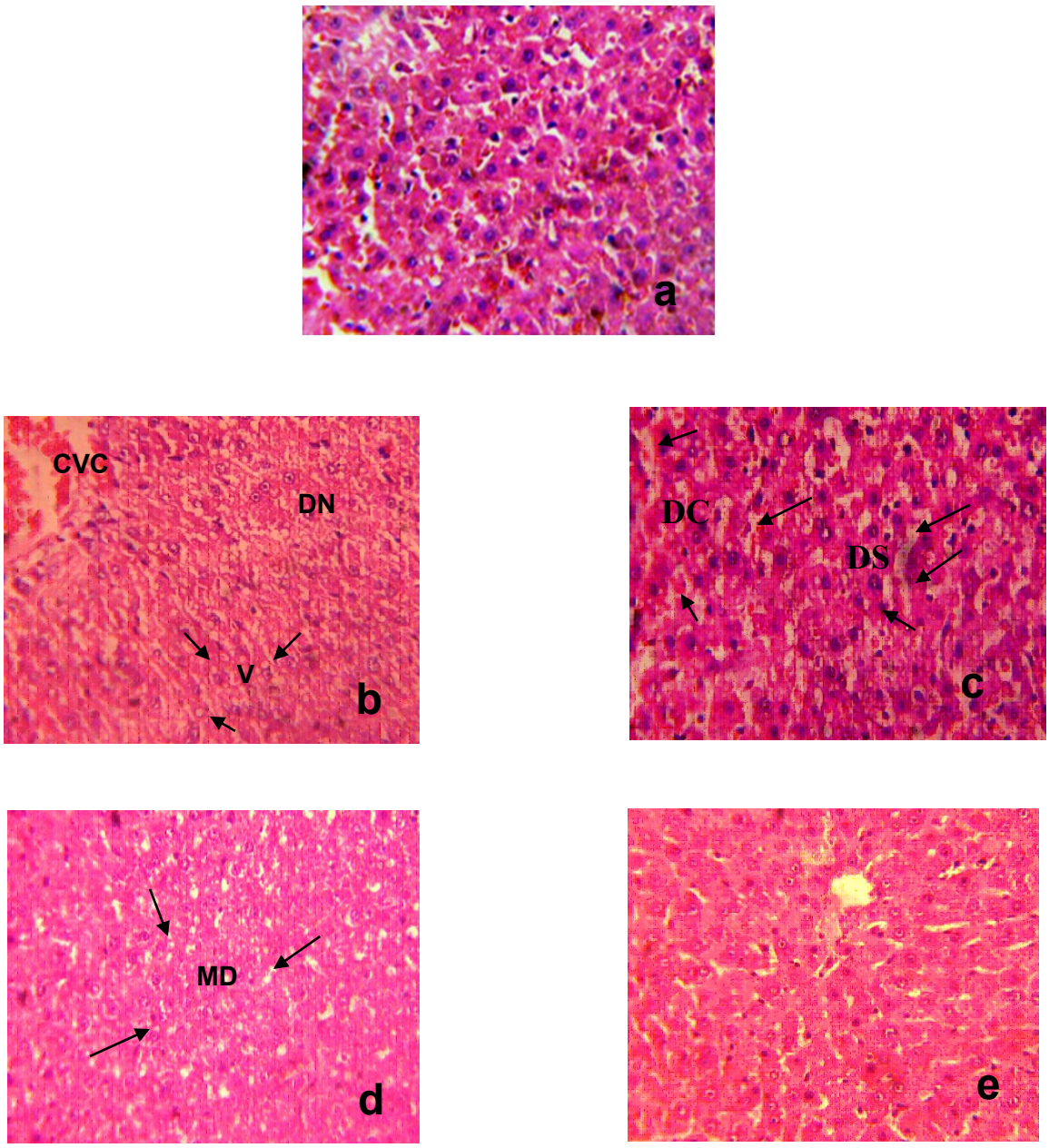

Fig. 15— Section of liver stained with Haematoxylin and Eosin (H & E × 40):

(a) NC

(b) CO administered group

DN– Diffuse necrosis
CVC – CentralVenous Congestion
V – Vacuolation

(c) SCO administered group

DS- Dilatation of sinusoids
DC- Diffused congestion

(d) VCO administered group

MD- Mild degeneration

(e) FO administered group

Normal hepatocytes

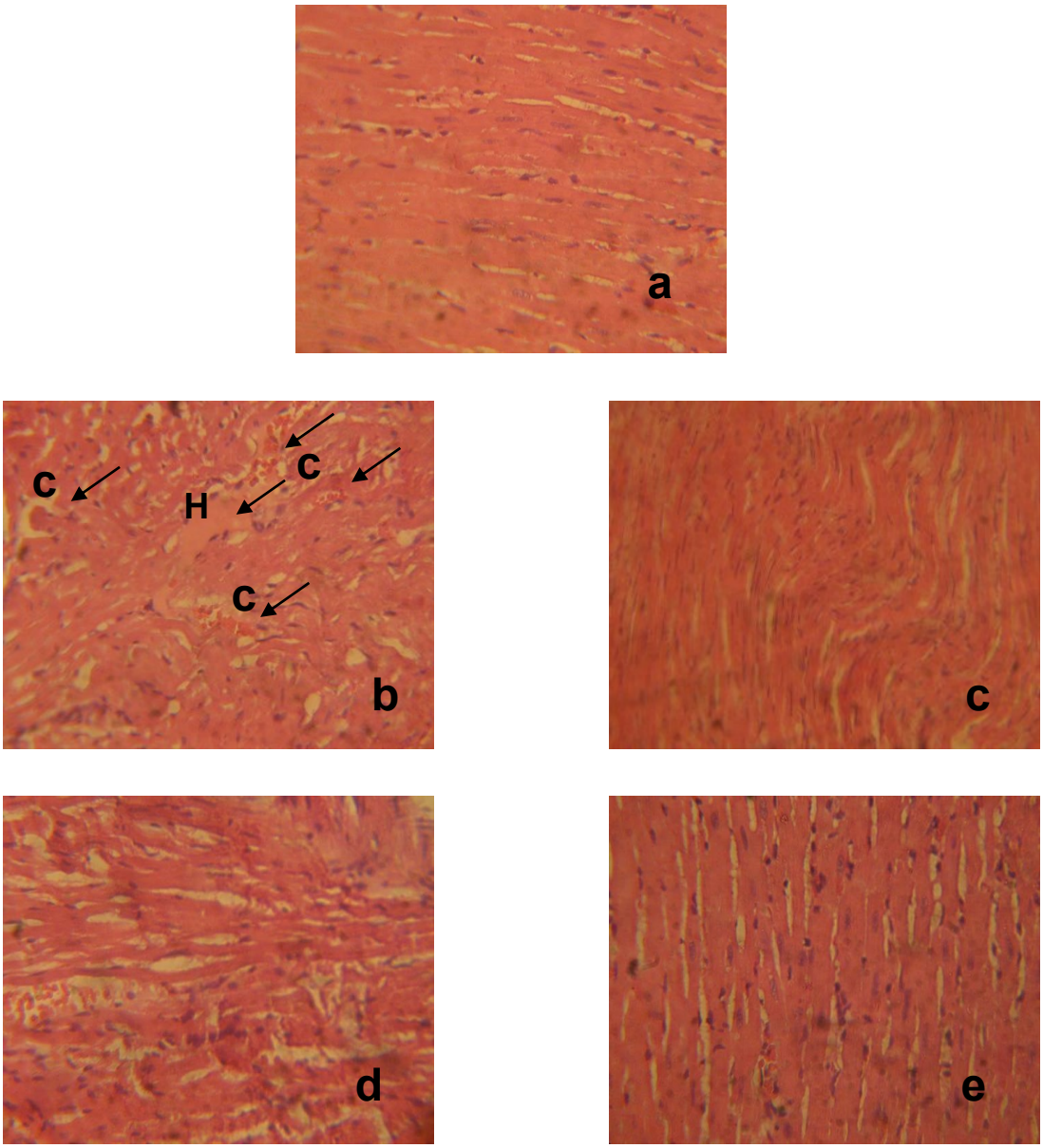

Fig. 16 — Section of heart stained with Haematoxylin and Eosin (H & E × 40):

(a) NC

(b) CO administered group

C– Congestion, H- Hyalinization

(c) SCO administered group

Regular cardiac fibres

(d) VCO administered group

Regular cardiac fibres

(e) FO administered group

Regular cardiac fibres

5. DISCUSSION

Lipid profile and antioxidant status in rats were evaluated by administering various preparations of coconut oil [copra oil (CO), seasoned coconut oil (SCO) and virgin coconut oil (VCO)] and fish oil (FO). Appropriate amount of fat required in daily diet is a topic of controversy. Even though, consumption of small amount of saturated fats is essential, high correlation has been suggested between excessive amount of saturated fat consumption and CHD (Clarke *et al.,* 1997). CHD is common in India and recently, an increase in the incidence of CHD was reported from the South Indian state of Kerala. Consumption of coconut kernal and oil which contains high amount of saturated fats, is believed to be strongly atherogenic and is attributed as a reason for the high incidence of CHD in Kerala (Kumar, 1997). This general belief made people of kerala to shift to alternate cooking oils like sunflower oil, which is rich in linoleic acid, an essential, ω-6 fatty acid (Sabitha *et al.,* 2009). Usually CO is made from coconut kernal dried either by exposing to very high temperature or sunlight for several days until most of the moisture content is removed. Exposure to sunlight or high temperature may inactivate minor components like tocopherols, tocotrienols and polyphenols (Wyatt *et al.,* 1998). Another preparation of CO is VCO, extracted by wet process directly from coconut milk under a controlled temperature retaining most of the unsaponifiable components like provitamin A, vitamin E and polyphenols (Nevin and Rajamohan, 2004).

Epidemiologic studies suggest that the consumption of a diet rich in marine lipids reduces the incidence of CHD (Kromhout *et al.,* 1985). Marine lipids, especially FO contains long-chain ω-3 polyunsaturated fatty acids (EPA and DHA), known to reduce plasma lipid level and platelet aggregation (Goodnight *et al.,* 1982). Though, there are studies that indicate a positive correlation between CO consumption and CHD, several reports contradict this proposal (Lipoeto *et al.,* 2001, Kritchevsky *et al.,* 2003, Nevin and Rajamohan,

2004 and Nicholls *et al.,* 2006). Since, CO is the major oil produced and consumed by the people of kerala and other southern states of India, the study taken up is relevant in the context to find out the actual role of CO in lipid metabolism.

5.1. BODY WEIGHT

There was no significant difference in body weight of rats administered with CO and SCO on day 90 when compared with normal rats. This might be due to high content of medium chain fatty acids (MCFAs) of CO, which on reaching hepatocytes do not require carnitine acyltransferase for their transport across the inner mitochondrial membrane (Foster, 1984). In mitochondria, these fatty acids are oxidized as a rapid source of energy and are considered to be less available for deposition in body fat (Tsuji *et al.,* 2001).

Long term administration of VCO was found to be effective in preventing substantial increase in body weight when compared with that of day 0 and control rats. In comparison with body weight of day 0, only 5% increase was observed in VCO fed rats whereas, about 20% and 40% increase was observed in control and FO fed rats respectively. These observations are in agreement with the reports of Takeuchi *et al.* (2008). They reported that high content of lauric acid, a MCFA, could prevent body fat accumulation since, it is rapidly absorbed and oxidized for energy purpose. VCO also contains high content of Lauric acid (50-57%) and the same mechanism might be responsible for the maintenance of body weight. It has been reported that replacing dietary long chain triacylglycerol (LCT) by medium chain triacylglycerols (MCTs) increased energy expenditure, decreased food intake, lowered body fat mass and decreased the size of fat depot in healthy over-weight men (Pierre *et al.,* 2002 and 2008). MCTs are readily hydrolyzed by lingual and gastric lipases to release MCFAs while, LCTs require intestinal lipase for cleavage (Bezard and Bugaut, 1986). Free MCFAs are absorbed readily into gastrointestinal cells, which diffuse rapidly into the portal circulation and after

reaching the liver, these are oxidized for energy purpose whereas, long chain fatty acids (LCFAs) require the detergent action of bile to enter the intestinal cell (Clark and Holt, 1968), reesterified into triacylglycerols (TAGs), packaged into chylomicrons and secreted into the lymph (Hashim *et al.,* 1964). Similar observations were also made by other researchers (Tsuji *et al.,* 2001 and St-Onge *et al.,* 2003).

FO administered rats showed a significant increase in body weight when compared to control rats and other oil administered groups. LCFAs including long chain polyunsaturated fatty acids (LCPUFAs) in FO absorbed from the small intestine are resynthesized into TAG in small intestinal mucosa cells, form chylomicrons, released into the circulation via the lymph vessels and transported to peripheral tissues for storage in fat depots (Pierre *et al.,* 2002 and Takeuchi *et al.,* 2008). Liu *et al.* (2009) also reported the incorporation of LCPUFAs into TAG in hepatocytes and this might be a reason for the increase in body weight of FO fed rats.

5.2. SERUM BIOCHEMISTRY

5.2.1. Total lipids (TL) and Triacylglycerol (TAG)

Initially a transient increase was observed in the level of serum TL, which decreased to that of control rats by day 90 in CO administered rats whereas, a significant rise was noticed in SCO fed rats throughout the period of study. Compared to SCO fed rats, CO administration significantly decreased the level of TL, which might be due to the decreased level of other lipid parameters such as TC, LDL and HDL in CO fed rats. Increased hepatic expression of apo C-III by MCTs in CO and apo C-III-mediated inhibition of catabolism of TAG-rich lipoproteins would contribute to a higher serum lipid and TAG concentrations (Wang *et al.,* 1998). Studies conducted by Heek and Zilversmit (1991) showed a substantial increase in TAG and apo B-100 in rabbits fed with CO diet than olive

oil diet, but the removal rate of apo B was same in both diet groups indicating that overproduction of hepatic TAG contributes to hypertriglyceridaemia. Plasma TAG level during saturated fat intake were found to be 34% and 63% higher compared to monounsaturated fatty acid (MUFA) and PUFA feeding respectively (Khosla and Hays, 1992). Chong *et al.* (1987) reported that changing from a diet containing corn oil (rich in PUFA) to CO significantly elevated plasma TAG, phospholipids, TC and all lipoproteins in Rhesus monkeys. The significant increase in TAG in CO fed rats is in agreement to the above studies.

Long term administration of VCO did not show any significant variation in the level of both TL and TAG from that of control rats, which might be due to the relative rate of synthesis and catabolism of these lipids. Similar observations were made by Nevin and Rajamohan (2004) on the level of serum TAG in VCO fed rats and suggested that this could be due to the quality difference of CO and VCO. VCO contains high content of polyphenols (80mg/100g oil) and minor constituents such as vitamin A and E compared to CO. Zhou *et al.* (2009) showed that oral administration of flavonoids (200 mg/kg), a polyphenol, for 28 days significantly reduced the level of serum total cholesterol and TAG levels. Similar results were reported by Isabel *et al.* (2006). They evaluated the effect of phenolic content of olive oil taken at a daily dose of 25 ml for 3 weeks on plasma lipid levels in humans and observed a decrease in the serum TAG level. Hypolipidaemic effect of flavonoids and phenolic compounds was also reported by Hakkim *et al.* (2007).

Administration of FO showed a transient increase in the levels of serum TL and TAG during the first half of the study and later by 3 months the level of TL decreased compared to basal level and in comparison with all other groups. However, after 90 days of oil administration, an increased level of serum TAG was observed when compared with the basal level, VCO fed and control rats but, a significant decrease was noted in comparison with CO and SCO fed rats. Lipid lowering effect is in accordance with the report of Frenoux *et al.* (2001). They

found lowered plasma lipid concentration in rats fed with a diet rich in PUFA for 10 weeks. Jenkins and Kramer (1990) also observed decreased levels of plasma total lipids, phosphatidylcholine, and cholesteryl esters in calves fed with a mixture of corn oil and FO diet either in the ratio 2:1 or 1:2 for a period of 14 days. Effect of FO on lipid concentrations in postprandial plasma has been ascribed to the constituent (n-3) PUFA (EPA and DHA), the active component, which are known to reduce both plasma lipid levels and platelet aggregation (Bergeron and Havel, 1997). Higher level of serum TAG might be due to an interaction between EPA (34.9%) and DHA (26.4%) present in FO on TAG metabolism. Harris (1997) reported a dose related response between omega-3 fatty acid intake and triglyceride lowering effect. Willumsen *et al.* (1993) showed that DHA at a dose of 1000 mg/kg b.w/day for 10 days did not inhibit the synthesis and secretion of TAG in the liver of rats, while, EPA administration at the same dose showed a hypotriglyceridaemic effect. An increase in peroxisomal β-oxidation and fatty acyl-CoA oxidase activity was observed with both EPA and DHA but, DHA did not exhibit lipid-lowering effect. Banerjee *et al.* (1992) conducted studies in rats using dietary FOs with different n-3 PUFA compositions, showed that feeding diets containing marine Hilsa FO decreased the levels of serum cholesterol, TAG and phospholipids while, chital oil (oil from fresh water fish) elevated plasma cholesterol level while TAG and phospholipid contents remained unaltered. The study strongly suggests that the hypolipidaemic effect depends on the composition of n-3 PUFA rather than on the total n-3 PUFA content of the dietary FO.

In contrast to the present findings, Maree *et al.* (2009) reported high serum TAG level in rats supplemented with FO diet compared to CO diet. Studies conducted by Nydahl *et al.* (1994) reported no significant difference in serum TAG concentration between control rats and rats fed with MUFA and PUFA diet. It has also been reported that hypotriglyceridaemic effect of FO depends on an interaction between dietary proteins and lipids. Demonty *et al.* (1998) showed that soy protein enhanced hypotriglyceridaemic effect of FO whereas, casein and to a

greater extent cod protein dampened it. Thus, the response to dietary FO in a given individual depends on a number of factors including the quantity and source of the marine lipid ingested, the degree to which other dietary fats are present and the underlying lipid phenotype of the individual (Ventura *et al.* 1989).

5.2.2. Total cholesterol (TC)

A transient increase was observed in serum TC level in CO fed rats, which decreased to the basal level by 90 days whereas, SCO administration showed a significant increase through out the period of study. Transient increase and then a decrease in the level of TC observed on CO administration suggest an adaptive mechanism to maintain the level either by enhancing the degradation of formed cholesterol and increasing its excretion through bile or its utilization by tissues for synthetic purpose. Cox *et al.* (1995) reported that CO diet showed a lesser effect in increasing serum TC than butter fat, in hypercholesterolaemic men and women. Difference between CO and SCO lies on the fat soluble components of seasoning ingredients such as, turmeric, red onion, mustard seeds and curry leaves and the temperature (60 – 70ºC) at which the seasoning has been carried out. Heat stability of CO is well known, which makes it an excellent cooking and frying oil (Bruce, 2005). Therefore, the chance for the formation of trans fatty acids is sparse. The reason for this varying effect between CO and SCO is not clearly understood. It might be due to the presence of phytosterols and squalene in SCO, which could upregulate cholesterogenesis either by indirect suppression of cholesterol absorption from small intestine or directly by serving as a precursor for cholesterol synthesis (Jones *et al*. 1994). Though there are reports on the hypocholesterolaemic effect of polyphenols (Khor *et al.,* 1998 and Akhthar *et al.,* 2007), Asai and Miyazawa (2001) reported that the yellowish polyphenolic pigment of turmeric, curcuminoid, had no hypocholesterolaemic effect in normal rats.

VCO intake also showed a trend similar to that of CO, which agrees with the reports of Nevin and Rajamohan (2004 and 2008). They observed that feeding of VCO supplemented diet significantly reduced serum TC level compared to CO and the effect was comparable with that of sunflower oil fed rats. It has been suggested that this might be due to the relative rate of synthesis and catabolism of serum lipids or due to the higher polyphenol content of VCO having hypocholesterolaemic effect (Khor *et al.,* 1998).

FO administration did not cause any significant variation in serum TC level throughout the period of study. Earlier workers reported similar observations (Nalbone *et al.,* 1988, Tripodi *et al.,* 1991, Banerjee *et al.,* 1992, Mortensen *et al.,* 1998 and Maree *et al.*, 2009). It has been suggested that hypocholesterolaemic effect of FO might be either due to the high affinity of LDL to liver plasma membranes (Tripodi *et al.,* 1991) or due to an increased rate of excretion of cholesterols and bile acids in the faeces (Ahmed *et al.,* 2006). Chen *et al.* (1987) reported that cholesterol absorption was lower in the presence of menhaden oil or FO concentrate than in the presence of oleic acid or corn oil. They suggested that the lower cholesterol in thoracic lymph might be due to reduced cholesterol absorption or digestion of TAGs in FO or due to higher sterol excretion.

Chitra *et al.* (2000) suggested that presence of eicosanoids especially, prostaglandins in FO, decreased serum TC level in rats by increasing the level of cAMP in platelets, lungs and adipose tissue which in turn decreased lipolysis at the adenylate cyclase site, acting through a Gi protein. Giudetti *et al.* (2003) showed that the mitochondrial tricarboxylate carrier (TCC) plays an important role in lipogenesis and rats fed with FO diet showed a marked decrease in TCC activity in liver mitochondria, when compared with CO diet. This might be due to a reduced gene expression of the carrier protein. Activities of lipogenic enzymes such as, acetyl-CoA carboxylase, fatty acid synthase (FAS), ATP-citrate lyase, stearoyl-CoA desaturase, malic enzyme, glucose 6-phosphate dehydrogenase, and the S14 protein, were greatly reduced by PUFA administration (Toussant *et al.,*

1981 and Clarke *et al.,* 1990). The n-3 PUFAs also play a crucial role as "fuel partitioners," where they direct fatty acids towards oxidation rather than storage as TAG. They act by upregulating the expression of genes encoding proteins involved in fatty acid oxidation while downregulating genes encoding proteins of lipid synthesis (Clarke, 2001). On the other hand, MUFA like oleate (C18:1, n-9) or saturated fatty acids like palmitate (C16:0) and MCFA (present in CO) do not inhibit either the activities or the expression of the lipogenic enzymes (Clarke and Abraham, 1998).

5.2.3. Serum lipoproteins

Administration of CO showed a transient increase in HDL level, which decreased to basal level by day 90. Level of LDL reduced significantly whereas, that of VLDL significantly increased throughout the period of study when compared with control rats. Transient increase in HDL level was similar with the findings of Carlson and Kottke (1991). They observed an increased level of plasma apo A-I and HDL-cholesterol in Watanable (WHHL) rabbits, fed with CO diet as well as a diet containing trilaurin for 3 days. It was also noted that withdrawal of these oils from diets immediately decreased the apo A-I levels. It has been suggested that lauric acid, the active component in CO, increased the apo A-1 levels by regulating its synthesis and secretion by intestinal mucosa. Chong *et al.* (1987) conducted studies in Rhesus monkeys and reported that changing from a diet containing corn oil (rich in PUFA) to CO significantly elevated all classes of plasma lipoproteins without altering the lipoprotein size, suggesting an increase in particle number. Increased hepatic apo A-I expression may contribute to higher levels of apo A-I and serum HDL in new born pigs fed with intermediate chain triglycerides, which is a component of CO (Wang *et al.,* 1998). Hostmark *et al.* (1980) reported that rats fed on diets containing CO showed significantly higher level of alpha-lipoproteins (HDL) compared to sunflower oil fed group.

Cox *et al. (*1995) showed significantly lower level of LDL in individuals on CO diet than on butter fat diet, which agrees with the present findings. Denke and Grundy (1992) compared liquid formula diets rich in lauric, palmitic and oleic acids and showed that both lauric and palmitic acids were associated with higher levels of LDL cholesterol when compared with oleic acid but the rise in LDL cholesterol on the high lauric acid diet was about two thirds of that on the high palmitic acid diet. CO also contains high level of Lauric acid (43%), which might have caused the decrease in LDL. Suma (1998) reported that CO consumption was beneficial in reducing LDL and increasing HDL when compared to groundnut oil in human.

Heek and Zilversmit (1991) observed significantly high content of VLDL in rabbits fed on a CO diet compared with olive oil diet, which is similar to the present findings. They suggested that this elevation in VLDL might be due to an increase in particle number rather than particle size, increased hepatic secretion of VLDL and a modest decrease in VLDL TAG clearance capacity. Similar observations were also made in human studies conducted by Cox *et al*. (1995)

SCO fed rats maintained high levels of HDL and LDL throughout the experimental period while VLDL showed a significant increase only by day 90. Beena *et al.* (1996) conducted studies in rats by supplementing curry leaves and mustard seeds (each 10%) to a standard diet with 20% CO for 90 days. They showed an increase in HDL, while LDL and VLDL levels were decreased and their observations partly agree with our findings. They suggested that polyphenols and flavonoids present in curry leaves and PUFA content of mustard seeds might be responsible for this effect. In the present study, curry leaves, mustard seeds, onion and turmeric were used only to season CO and the strained oil was administered to rats, which might contain only fewer amounts of these active principles. Increase in levels of LDL and VLDL might be due to low content of the active principles or an antagonistic effect exerted by the low content. Various other researchers reported on the hypolipidaemic and HDL increasing effects of

curry leaves (*Murraya koenigii*), mustard seeds (*Brassica juncea*), turmeric (*Curcuma longa*) and onion (*Allium cepa*) when fed along with diets or as alcohol extracts (Hussain, 2002, Kesari *et al.,* 2007 and Yamamoto and Yasuoka , 2010). Sabitha *et al.* (2009) compared the lipid profile of persons, who were consuming CO with those consuming sunflower oil as cooking medium. They found no significant changes in lipid profile between CO and sunflower oil consuming groups. Though not statistically significant, HDL concentrations were higher in CO group compared to sunflower oil group. The findings indicated that habitual use of CO as a cooking medium has no specific role in hyperlipidaemia.

HDL level in VCO fed rats was similar to that of SCO fed rats. There was no significant variation in the level of LDL during the first half of study period while, it decreased significantly by 90 days. However, VLDL level did not show any significant variation during the entire period of study. This is in agreement with the findings of Nevin and Rajamohan (2004). They observed increased levels of serum HDL and decreased levels of phospholipids, LDL and VLDL in rats fed with VCO diet as compared to CO diet. The lower lipid levels in serum accompanying VCO feeding might be due to the relative rate of synthesis and catabolism of these lipids. It could also be attributed to the presence of biologically active unsaponifiable components viz., vitamin E, provitamin A, polyphenols and phytosterols. Similar observations were also reported by Isabel *et al.* (2006). Their study in human beings, revealed a reduction in total cholesterol/ HDL ratio and an increase in the level of serum HDL by the phenolic content of olive oil. Zhou *et al.* (2009) reported that oral administration of flavonoids significantly increased the level of serum HDL.

FO administration did not cause any significant variation in the levels of serum HDL and VLDL whereas, a significant decrease was observed in the level of serum LDL throughout the experimental period. Similar observation was reported in the level of serum LDL when rats were supplemented with a diet rich in FO (Maree *et al.,* 2009). Reduction in LDL level might be due to an increased

LDL receptor activity in hepatic tissue and the lipid in FO could have affected the LDL receptor pathway by altering the composition and, perhaps, the physical properties of hepatic membranes. It could also be due to a reduction in the rate of LDL-cholesterol production and by increasing the rate of receptor dependent LDL transport (Ventura *et al*., 1989). Tripodi *et al*. (1991) also reported a high binding affinity of LDL to liver plasma membranes when rats were fed with FO rich diet.

Vlijmen *et al.* (1998) reported that feeding mice with an FO rich diet decreased the level of serum VLDL without affecting the levels of LDL and HDL, whereas, the present study showed a reduction in serum LDL with no effect on the levels of VLDL and HDL. Haug and Hostmark (1987) showed that the levels of plasma TAG, cholesterol and phospholipids as well as the lipoprotein catabolizing enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL) were decreased in rats fed with FO diet for 4 weeks and suggested that this could be due to a fall in VLDL and HDL_2, with less consistent changes in LDL and HDL_3. The decrease in LPL and HL activity of FO fed rats might be an adaptive response to the low concentration of the substrates for these enzymes (LPL - degrades VLDL in peripheral tissues and HL- a key enzyme for hepatic uptake of HDL_2). Nestel *et al*. (1984) reported that FO rich diet lowered the plasma VLDL lipids and apo B concentrations profoundly in normal subjects.

HDL/LDL ratio observed in different groups showed that CO, VCO and FO administration increased the ratio and maintained at a higher level throughout the period of study, however, highest ratio was achieved by VCO administration. Observation after 90 days revealed significantly decreased level of LDL and no change in HDL in CO and FO fed rats whereas significant increase in HDL level and decrease in LDL level in VCO fed rats resulted in high HDL/LDL ratio. Though, SCO administration did not cause a considerable increase in HDL/LDL ratio, the value did not decrease from the basal level.

5.3. WEIGHT OF LIVER AND HEART

Significant increase was observed in the weight of liver and heart in CO fed rats while, SCO administration decreased liver weight and maintained the weight of heart similar to that of control rats. Increased weight of both the organs in rats on CO rich diet could be attributed to oxidative damage and resultant inflammation, which is evident from the level of lipid peroxides. Very high content of TAG observed in both the tissues also contribute to the rise in weight. Histopathological examination also correlated these observations. Zulet *et al*. (1999) reported that a hypercholesterolaemic diet rich in CO significantly increased liver weight, accompanied by an increase in the content of cholesterol in liver and aorta. Levels of lipid peroxides and TAG in both the tissues were maintained in SCO administered group and did not reveal any tissue damage or fat deposition, which might have contributed to the observations on weight of these organs. Microscopic examination of the tissues also supported these findings with the exception of slight changes in hepatic tissue.

Long term administration of VCO did not cause any significant variation in the weight of both the organs, which is supported by the biochemical parameters and histological observations. VCO did not cause any significant variation in the levels of tissue total cholesterol and TAGs. Microscopic examination also revealed a normal histological architecture of both the tissues. Feeding FO caused a significant increase in weight of both the vital organs. This could be attributed to oxidative damage and resultant inflammation of tissues, which is evident from the increased level of lipid peroxidation. Microscopic examination showed a normal histological architecture of both the tissues since, it is not possible to visualize such changes on membranes by ordinary microscopic examination. The present finding is in accordance with Parrish *et al.* (1991). They showed that adipose tissue (epididymal and perirenal) was the only tissue whose mass decreased after FO supplementation, whereas mass of liver and spleen

increased. Similarly, Park and Park (2009) also showed a significantly lower abdominal fat and higher weight of liver and kidney in rats fed with FO diet.

5.4. TISSUE BIOCHEMISTRY

5.4.1. Total cholesterol (TC) and Triacylglycerol (TAG)

Both CO and SCO administration increased TC level in liver and heart. Level of TAG increased to a great extent in both the tissues of CO fed rats while it was maintained to that of control rats in SCO fed group. Higher level of liver cholesterol in rats fed with CO was also reported by other researchers (Demonty *et al.,* 1998 and Zulet *et al.*, 1999). This might be due to an increased HMG CoA reductase activity in the liver of rats fed with saturated fatty acids. Asai and Miyazawa (2001) showed that rats fed with a diet supplemented with 1% curcuminoids significantly increased hepatic acyl-CoA oxidase activity and lowered the levels of triacylglycerol and cholesterol in liver. They suggested that lipid lowering effect of curcuminoids might be due to alterations in fatty acid metabolism. Presence of curcuminoids, the phenolic yellowish pigments of turmeric in SCO, might have played a role in reducing the liver TAG level.

Long term administration of VCO caused a significant reduction in cholesterol in both liver and heart. However, TAG level was significantly increased in heart while it was maintained to that of control rats in liver. Nevin and Rajamohan (2004) reported reduced levels of cholesterol and TAG in liver, heart and kidney of rats fed with VCO for 45 days. They suggested that it could be due to the reduced activity of HMG CoA reductase, the rate limiting enzyme in cholesterol biosynthesis and also due to the relative rate of synthesis and oxidation of fatty acids in liver. Moreover, reduction in the activity of the lipogenic enzyme, glucose-6-phosphate dehydrogenase, decreased lipogenesis.

Rats fed with FO significantly decreased the level of liver cholesterol while, the level of cholesterol in heart and TAG in both the tissues were similar to

that of control rats. This is in accordance with the findings of Chitra *et al.* (2000). They showed that rats fed with FO diet lowered tissue cholesterol and TAG levels due to the presence of eicosanoids especially, prostaglandins in FO, increasing the level of cAMP in platelets, lungs and adipose tissue which in turn decreased the lipolysis at the adenylate cyclase site, acting through a Gi protein. Ahmed *et al.* (2006) also reported significantly lower level of liver cholesterol in rats fed with FO diet compared to soybean and palm oil diets and suggested that this decrease could be mainly due to an increased rate of excretion of cholesterol and bile acids in faeces. Similar observations were also made by Garg *et al.* (1989). Jenkins and Kramer (1990) conducted experiments in calves by feeding corn oil or corn oil and FO concentrate and reported that the PUFA enriched feed had no effect on the concentration of major phospholipids in liver, heart, muscle, and brain. Cardiac protein, TAG and cholesterol concentrations were unaffected by feeding salmon oil for a period of 2 months in rats (Nalbone *et al.,* 1988). Fatty acid synthase (FAS) activity was markedly lower in the liver but not in the adipose tissues of rats fed with FO diet. The decrease in visceral fat in rats fed with FO diet was attributed to increased lipid mobilization rather than reduced lipid storage (Caso *et al.,* 2003). Dietary cholesterol and/or ω3 fatty acids of linseed oil or FO did not have any appreciable effects on heart and adipose tissue cholesterol concentrations. It is possible that entry and exit of cholesterol is tightly regulated in heart and adipose tissue as the cholesterol content remained unaffected even after a high load of dietary cholesterol (Garg *et al.,* 1989).

5.4.2. Lipid peroxides (LP) and reduced glutathione (GSH)

CHD is considered as an inflammatory disease and ample evidence is now available to suggest that oxidative stress either contribute or aggravate the process of atherosclerosis (Ronald, 1999 and Chen and Jawahar, 2004). Oxidation of lipid molecules of biological membranes causes membrane damage resulting in the development of several physiological and pathological disorders (Chaturvedi, 2007). Malondialdehyde (MDA), a secondary product of lipid peroxidation, is

known to cause cross-linkage of membrane components containing amino groups that makes the membrane fragile. Lipid peroxidation causes serious damage to cell membranes thereby leakage of intracellular enzymes resulting in loss of cell function and death. Estimation of thiobarbituric acid reactive substances (TBARS) is the diagnostic index of lipid peroxidation and tissue injury due to oxidative stress (Blaha *et al.,* 2004). Primary products of lipid peroxidation can undergo carbon–carbon bond cleavage via alkoxyl radicals in the presence of transition metals, giving rise to the formation of short-chain, unesterified aldehydes (Kawai *et al.,* 2003). Glutathione is a substrate, scavenging toxic intermediates of incomplete oxidation. Thus the total glutathione concentration as well as its ability to maintain glutathione in reduced state is an important defensive mechanism against oxidative stress (Pricilla, 1995).

A significant increase was observed in the level of LP and GSH in both liver and heart of CO fed rats. Feeding SCO did not show any significant effect in the level of LP in both the tissues and GSH in liver of rats whereas, the level of GSH increased significantly in heart when compared with control rats. It could be suggested that rise in GSH level in both the tissues of CO fed rats along with a rise in LP is a compensatory mechanism to scavenge the free radicals generated. This is in accordance with the reports of Nevin and Rajamohan (2006). They observed an increased TBARS level as well as an increased activity of the antioxidant enzymes, catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase in liver, heart and kidney of rats fed with CO diet compared to ground nut oil diet. Antioxidant enzymes are capable of scavenging free radicals and generating GSH. Feeding SCO appears to be hepato protective, at least partly, which could be attributed to the active principles, of the added ingredients in SCO, such as flavonoids and other polyphenols. These active principles are strong antioxidants capable of scavenging free radicals. Flavonoids and other polyphenols have been reported to exert a variety of biological actions such as free radical scavenging, chelation of metal ions, modulation of enzyme activity, signal transduction, activation of transcription factors and gene

expression (Rice-evans *et. al*., 1995, Natarajan *et al.,* 1996, Bito *et al.,* 2000 and Blaha *et al.,* 2004). Quercetin, one of the most abundant flavonoids of onion (*Allium cepa* L.) has strong antioxidant activity (Fremont *et al.,* 1998 and Duarte *et al.,* 2001). Arulselvan and Subramanian (2007) reported the antioxidant activity of flavonoids and other phenolics of curry leaves (*Murraya koenigii*), which decreased TBARS level and increased enzymatic and non-enzymatic antioxidant activities in diabetic rats. Mustard oil has also been shown to have antioxidant activity, which increased superoxide dismutase and significantly decreased lipid peroxidation. It has been attributed to the ability of ω 3 fatty acids in mustard oil to inhibit inflammatory pathways and suppress the expression of large number of genes related to lipid metabolism. It shows that not just PUFA, but the type of PUFA present in the dietary oil used is important (Benson and Devi, 2009).

Administration of VCO decreased the level of LP in both liver and heart though, not significantly different from that of control rats. However, a significant decrease was observed in the level of GSH in both the tissues. Nevin and Rajamohan (2006) made similar observation on the level of LP and related enzymes, such as, catalase and superoxide dismutase in tissues of rats fed with a diet containing VCO, while their observations on GSH related enzymes were contradictory to the present observation. They reported significantly higher levels of Glutathione peroxidase and glutathione reductase in these tissues. High content of unsaponifiable components such as, vitamin E and polyphenols might contribute to the beneficial effect of VCO. Even though fatty acid analysis of VCO and CO indicated same amount of saturated fatty acids (Nevin and Rajamohan, 2008), the positive effects of VCO could be due to the difference in absorption, transport and catabolism of the constituent fatty acids. Reduction in GSH content of both the tissues is not clearly understood and it might be related to the reduced oxidative stress.

Rats fed with FO diet showed significantly increased level of LP in both the tissues. Level of GSH increased significantly in tissues of heart whereas, it did not vary significantly in liver, when compared with control rats. Increased level of lipid peroxidation was also reported by Labbe *et al.* (1991). They showed an increased tissue TBARS level in rats fed with a diet rich in n-3 fatty acids for 16 weeks, which occurred in conjunction with increased incorporation of n-3 fatty acids in tissues and decreased activity of the protective enzymes, superoxide dismutase and glutathione peroxidase. Accumulation of products of lipid peroxidation in heart, skeletal muscle and mammary glands was also reported in rats on FO diet for 4 weeks (Gonzalez *et al.,* 1992). It has also been shown that incorporating high doses of EPA and DHA (1500mg/day/kg b) in rat's diet resulted in elevated levels of MDA in plasma and liver (Vaagenes *et al.,* 1998). Addition of antioxidants such as, vitamin E (500 mg/kg b.w) in FO supplemented diets decreased the levels of TBARS and protein carbonyls in tissues of rats except hepatic lipid peroxidation, which remained high when compared with soy oil–vitamin E diet (Sen *et al.*, 1997). Similar observations were made by Mete *et al.* (1999), when rats were fed with FO rich diet and FO diet supplemented with vitamin E. A positive correlation was found between dietary vitamin E and plasma vitamin C, erythrocyte GSH and liver glutathione peroxidase. Studies conducted by Meydani *et al.* (1987) compared the effect of α-tocopherol (potent antioxidant) in FO, CO and corn oil diets on plasma and tissue lipid peroxidation and suggested that either reduced level of α-tocopherol or its rate of absorption from the intestine, might be one of the reasons for increased lipid peroxidation observed in rats on FO diet.

Comparing the level of LP in liver and heart with that of control rats revealed that the level in liver increased nearly double while in heart the increase was only about one and a half times whereas, the content of GSH in heart nearly doubled and in liver, it was similar to that of control rats. It shows that the oxidative damage was higher in liver than heart. Glutathione functions as a direct free radical scavenger and stabilizes membrane structure through the removal of

products of lipid peroxidation. As an adaptive response to increased oxidation, GSH content would have been increased in both the tissues while an insignificant increase in the level of GSH and a significant rise in the level of lipid peroxides in liver, suggest that there might have been a rapid utilization of the newly synthesized/transported GSH for the elimination of reactive oxygen species (ROS) and quenching the products of lipid peroxidation. Several other researchers showed that supplementation of FO diet with vitamin E decreased oxidative stess and increased the content of GSH and activated the enzymes, such as, Glutathione reductase and glutathione peroxidase and glucose 6 phosphate dehydrogenase concerned with GSH production (Ibrahim *et al.*,1997, Sen *et al.*, 1997 and Mete *et al.*, 1999).

5.4.3. Liver glycogen

Administration of CO and SCO significantly reduced liver glycogen content while the decrease was about 35% and 70% in CO and SCO fed rats respectively, compared to control rats. Moreover, CO fed rats were only hypertriglyceridaemic while SCO fed rats showed hyperlipidaemia (significantly high levels of TL, TC and TAG). Variation in food intake due to hypertriglyceridaemia and hyperlipidaemia might have resulted in varying degree of reduction in the content of liver glycogen in CO and SCO fed rats respectively. Zulet *et al.* (1999) reported low serum glucose level, about 50 % reduction in serum insulin level and decreased content of liver glycogen in rats fed with a high fat diet enriched with CO and cholesterol. They also observed reduction in the level of feed intake in hypercholesterolaemic rats. It has been suggested that the diet, impaired signal transduction mechanisms in pancreatic beta cells followed by reduced insulin secretion, which led to low content of liver glycogen.

Rats fed with VCO showed a liver glycogen content similar to that of control rats. This might be due to the high content of flavonoids and other polyphenols in VCO. Flavonoids increase glucose stimulated insulin secretion

from islet cells, which enhances glycogenesis by the activation of the glycogen synthase system (Pinent, 2008).

Liver glycogen level in the FO fed rats was similar to that of SCO fed group. This is in accordance with the finding of Rustan *et al.* (1993). They observed a decrease in liver and muscle glycogen content by 26 and 41 % respectively, in rats fed with FO diet when compared to lard rich diet. It was also noted that in FO fed rats the serum insulin level decreased while there was an increase in respiratory quotient (RQ) showing increased carbohydrate utilization.

5.5. HISTOPATHOLOGICAL EXAMINATION OF LIVER AND HEART

Liver and heart of CO fed rats showed central venous congestion, diffuse necrosis and vacuolation of hepatocytes while, lesions in heart were characterized by congestion and moderate hyalinization of some of the heart fibres, amidst healthy cardiac fibres. Observations made in hepatic tissue agreed with the findings of Maree *et al.* (2009). They noted large lipid depositions in the liver of rats fed with CO rich diet. Microscopic examination of liver of rats fed with SCO showed normal hepatocytes, with dilatation of sinusoids and diffuse congestion whereas, heart tissue revealed regular fibres. This might be attributed to the presence of antioxidants (flavanoids and other polyphenols) in SCO. Asai *et al.* (1999) conducted experiments in mice and showed that curcuminoids present in dietary turmeric prevented the accumulation of TAG in liver of mice and fatty liver disease associated with hyperlipidaemia and obesity.

A normal histological architecture of both liver and heart were exhibited by VCO and FO administered rats except for a mild degeneration of hepatocytes in the former group. This could be attributed to the normolipidaemic effect of these oils. This is in accordance with the findings of Maree *et al.* (2009). They reported similar observations in liver and kidney of rats fed with FO supplemented diet.

6. SUMMARY

The present study was undertaken to assess the effect of various preparations of coconut oil (copra oil, seasoned coconut oil and virgin coconut oil) and fish oil on lipid profile and antioxidant status in rats.

Experiments were carried out in 30 adult male Wistar rats weighing 180-220 g, which were randomly divided into five groups comprising six animals each. Group, G1 served as normal control (NC), G2 administered with coconut oil (CO), G3 with seasoned coconut oil (SCO), G4 with virgin coconut oil (VCO) and G5 with fish oil (FO). Oils were administered orally to rats at a dose of 16.4g/kg body weight per day using an orogastric tube.

Duration of experiment was 90 days. Weight of all rats was recorded and blood samples were collected on day 0 (just before oil administration), 45^{th} and 90^{th} day of oil administration. All the animals were euthanized on day 90. Liver and heart were separated and weighed.

Biochemical parameters viz., triacylglycerol (TAG), total lipids (TL), total cholesterol (TC), HDL, LDL and VLDL were estimated in serum. Tissues were analyzed for TC, TAG, lipid peroxides (LP), reduced glutathione (GSH) and liver glycogen. Representative samples of liver and heart tissues were subjected to histopathological examination.

CO and SCO administered rats maintained the body weight similar to that of NC. However, the body weight of SCO administered rats was significantly ($P< 0.05$) lower than that of CO administered ones. VCO administration significantly ($P< 0.05$) decreased while FO administration significantly ($P< 0.05$) increased the body weight when compared with all other groups. When compared with body weight on day 0, VCO administered rats showed only 5% increase in weight, while there was 20% increase in NC and about 40% increase in the FO administered group.

Administration of both CO and SCO resulted in a considerable increase in the levels of TL on day 45 but it returned to normal level in CO fed group by day 90. SCO group also showed a considerable reduction but the level was significantly ($P< 0.05$) higher than normal level on day 90. Both CO and SCO administered group showed significant ($P< 0.05$) increase in TAG level throughout the experimental period. Long term administration of VCO did not cause any variation in the levels of total lipids and triacylglycerol and were similar to that of NC rats. FO administration decreased the level of TL and increased the level of serum TAG when compared with NC rats. When compared with CO and SCO administered rats FO administration showed a significantly ($P< 0.05$) lower level of serum TAG.

Administration of CO significantly ($P< 0.05$) increased TC and HDL during the first half of the study period, while the levels of both decreased and returned to normal level during the second half. Long term administration of SCO resulted in a significant ($P< 0.05$) increase in both TC and HDL, while VCO intake increased only HDL keeping the level of TC normal. FO administration did not cause any variation in the levels of TC and HDL and they remained at the basal level throughout the period of study.

CO administration significantly ($P< 0.05$) reduced the level of LDL, whereas increased the level of VLDL throughout the period of study. Administration of SCO for three months significantly ($P< 0.05$) increased the level of both LDL and VLDL. Long term administration of VCO and FO resulted in a significant ($P< 0.05$) reduction in the level of LDL but the level of VLDL was not much affected by these treatments.

The ratio of HDL/LDL of the different groups showed that CO, VCO and FO administration increased the ratio significantly ($P< 0.05$) and was at a higher level throughout the period of study. This was because CO and FO maintained the level of HDL and reduced that of LDL. However, the highest ratio was achieved by VCO administration as it caused an increase in both HDL and LDL. Though,

SCO administration was not effective in increasing HDL/LDL ratio, the value did not decrease from the basal level.

Administration of CO and SCO significantly ($P< 0.05$) increased the level of TC in both liver and heart, while TAG increased in CO administered group and a value similar to NC was observed in SCO fed group in both tissues. VCO administration significantly ($P< 0.05$) decreased TC in both tissues when compared with NC rats. However, VCO increased the level of triacylglycerol in heart while the level was similar to that of NC in liver tissue. Administration of FO significantly ($P< 0.05$) decreased the level of TC in liver, while TC in heart and TAG in liver and heart were similar to that of NC rats.

The levels of LP and GSH increased significantly ($P< 0.05$) in liver and heart in CO administered rats whereas, in SCO administered group LP and GSH did not differ significantly except for a significant ($P< 0.05$) increase in heart GSH, when compared to NC. Administration of VCO decreased LP in both tissues, though not significant while, a significant ($P< 0.05$) reduction was observed in GSH content of both tissues. Level of LP increased maximally in both the tissues of FO group whereas a corresponding rise in GSH content was noted in heart but in liver it was similar to that of NC.

CO administration significantly ($P< 0.05$) decreased liver glycogen level while a drastic reduction in liver glycogen was noted in both SCO and FO groups, when compared with NC. Compared with normal rats liver glycogen in VCO administered group was similar to that of normal rats

CO and FO administration significantly ($P< 0.05$) increased the weight of both the vital organs (liver and heart). VCO administration did not cause any significant variation in the weight of both the organs while SCO administration decreased liver weight but the weight of heart was not much affected.

Hepatocytes of CO administered group showed diffuse necrosis with vacuolation and central venous congestion and lesions in heart were characterized by congestion and moderate hyalinization of some of the heart fibres, amidst healthy cardiac fibres. Normal histological architecture of heart tissue and dilatation of sinusoids and diffuse congestion with normal hepatocytes were observed in SCO group. Tissues of both liver and heart exhibited normal histological architecture in VCO and FO administered groups except for mild degeneration of hepatocytes in the former group.

Based on the above observations the following conclusions could be made:

1. Even though, serum TAG and VLDL levels increased, prolonged CO administration has no adverse effect on the serum levels of TL, TC, HDL and body weight. In fact, it decreased serum LDL level and increased the HDL/LDL ratio, which is a favourable factor in preventing CHD. In vital organs like liver and heart, administration of CO resulted in oxidative damage, high TAG level and low liver glycogen content. Histopathology changes concur with these observations.

2. SCO, mainly used by the people of Kerala while cooking, increased serum TL, TC, TAG, HDL, LDL and VLDL but have no effect on body weight. HDL/LDL ratio was maintained to the basal level. Absence of oxidative damage in liver and heart, increased content of heart GSH, are positive sign of amelioration of oxidative stress in the tissue. TC level increased with no change in TAG level in both the tissues and low liver glycogen content. Histopathology is also in tune with these observations.

3. VCO, a commercially available expensive preparation compared to CO, did not show any adverse effect on serum TL, TC, TAG and VLDL and decreased body weight than normal rats. Levels of HDL and LDL increased and decreased respectively thereby resulted in a substantial increase in HDL/LDL ratio, which is a favourable factor in preventing CHD. In liver, TC decreased and the levels of TAG, Glycogen, LP and GSH were unaffected. In

heart, TC, LP and GSH were decreased and TAG was increased. Histopathology also correlates with these observations.

4. FO, rich in polyunsaturated fatty acids (DHA and EPA), had no undesirable effects on serum lipid profile except for increased level of TAG and body weight. It decreased the levels of TL, LDL and increased HDL/LDL ratio. Oxidative damage was evident by increased level of LP in tissues and in heart GSH level also increased as a compensatory mechanism. No adverse effect on tissue lipid profile but a drastic reduction was observed in liver glycogen content.

It could be suggested that the effects of VCO and FO are congenial in preventing CHD and long term consumption of these oils would not cause any adverse effects on serum and tissue lipid profile, except for oxidative stress by FO. CO and SCO revealed some adverse effects, but it is worth mentioning that the dose of oil administered (16.4 g/kg body weight i.e. 30 kg/head/year) was very high, which stands in between per capita world average consumption (17.8 kg/head/year) and the consumption of developed western world (44 to 48 kg/head/year). Moreover this is more than double the per capita coconut oil consumption in Kerala (14 kg/head/year). Therefore, consumption of CO and SCO at a lower dose or the per capita consumption in Kerala as stated above might not induce adverse effects as pointed out in the present study.

Even though many studies are available on the affect of consumption of raw coconut oil, especially mixed with feed, studies on consumption as per se oil administration as well as administration of heated/seasoned CO is scanty. Since, coconut oil is consumed mainly after cooking, the effect of heat and the seasoning ingredients on coconut oil have to be further explored. Moreover, the dose fixed in this study was very high compared to the present consumption, further studies have to be taken up using varying doses of coconut oil as well as the effect of heating/seasoning on CO.

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FFECT OF COCONUT OIL AND FISH OIL ON LIPID PROFILE AND ANTIOXIDANT STATUS IN RATS

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ABSTRACT

The study was conducted to evaluate the effect of various preparations of coconut oil (copra oil, seasoned coconut oil and virgin coconut oil) and fish oil on lipid profile and antioxidant status in rats.

Adult male Wistar rats (180-220 g) were randomly divided into five groups and administered with oils as follows: G1 – normal control (NC), G2 – copra oil (CO), G3 – seasoned coconut oil (SCO), G4 - virgin coconut oil (VCO) and G5 – fish oil (FO).

Oils were administered orally to rats at a dose of 16.4g/kg body weight per day using an orogastric tube for a period of 90 days. Blood samples were collected on day 0 (before oil administration), 45 and 90 and serum was separated. Body weight was also recorded on the above days. Animals were euthanized on day 90; liver and heart were separated and weighed.

Serum were analyzed for biochemical parameters viz., TAG, TL, TC, HDL, LDL and VLDL and tissues for TC, TAG, LP and GSH. Liver glycogen was also estimated. Representative samples of liver and heart tissues were subjected to histopathological examination.

Administration of CO and SCO for 3 months did not show any significant variation in body weight. VCO administration significantly ($P< 0.05$) decreased whereas, FO administration significantly ($P< 0.05$) increased the body weight, when compared to NC. Serum TAG and VLDL were significantly ($P< 0.05$) increased while, LDL was decreased by CO administration. Serum TL, TC and HDL were similar to that of NC. SCO administered rats showed significantly ($P< 0.05$) higher levels of TL, TC, TAG, HDL, LDL and VLDL. VCO administration significantly ($P< 0.05$) increased the level of HDL and decreased LDL. Moreover, VCO administered rats did not show any significant variation in the levels of TL, TAG, TC and VLDL. Administration of FO showed the levels of TC, HDL and VLDL similar to that of NC, but TAG increased significantly ($P< 0.05$) and TL

and LDL showed a significant ($P< 0.05$) reduction. When HDL/LDL ratio was compared, CO, VCO and FO administered rats showed higher values when compared to NC. SCO administered rats, showed a value similar to that of day 0 but VCO administered group showed the highest value.

Administration of CO and FO significantly ($P< 0.05$) increased the weight of liver and heart while VCO administration did not show any significant variation from that of NC. SCO significantly ($P< 0.05$) decreased weight of liver whereas, weight of heart was similar to that of NC. Level of liver glycogen decreased significantly ($P< 0.05$) in all oil administered groups except VCO, which showed a level similar to that of NC. Administration of CO significantly ($P< 0.05$) increased the level of TC in heart and TAG in both the tissues, while TC in liver was similar to that of NC. SCO administration significantly ($P< 0.05$) increased TC whereas, the TAG levels were similar to that of NC in both the tissues. VCO consumption significantly ($P< 0.05$) decreased the level of TC in both the tissues, while it increased TAG in heart without affecting liver TAG. FO administration did not show any significant variation in the level of TC and TAG in heart, but it significantly ($P< 0.05$) decreased the level of TC without affecting liver TAG. Administration of CO and FO significantly ($P< 0.05$) increased the levels of LP and GSH in both the tissues except for liver GSH in FO group, which was similar to that of NC. Feeding SCO maintained the level of LP in both the tissues, while GSH increased significantly ($P< 0.05$) in heart while its level in liver was unaffected. VCO intake significantly ($P< 0.05$) reduced the level of both LP and GSH in heart, but maintained the normal level in liver.

Hepatocytes of rats administered with CO showed diffused necrosis with vacuolation and central venous congestion and lesions in heart were characterized by congestion and moderate hyalinization. SCO administered rats showed dilatation of sinusoids and diffuse congestion of liver but histological architecture in heart tissue was normal. VCO and FO administration exhibited normal histological architecture of both liver and heart except for a mild degeneration of hepatocytes in VCO consumed group.

Based on the above observations it could be suggested that long term consumption of VCO and FO might not cause any adverse effects on serum and tissue lipid profile except for oxidative stress by FO. CO and SCO feeding revealed some adverse effects, but it is worth mentioning that the dose of oil administered (16.4 g/kg body weight, i.e. 30 kg/head/year) was very high, which stands in between per capita world average consumption (17.8 kg/head/year) and consumption of developed western world (44 to 48 kg/head/year), also which is more than double the per capita coconut oil consumption in Kerala (14 kg/head/year). Therefore, consumption of CO and SCO at a lower dose or the per capita consumption in Kerala as stated above might not elicit adverse effects as observed in the present study.