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ASSESSMENT OF VARIOUS LIPID RICH DIETS FOR THE INDUCTION OF DYSLIPIDEMIA IN ANIMAL MODEL "AN EXPERIMENTAL STUDY"

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ABSTRACT

Objectives: The objective of this study was to evaluate various lipid rich diets for the induction of dyslipidemia in animal model.

Methods: This was an experimental study conducted on 42 Wistar albino rats. Animals were randomly divided into 7 groups on the basis of administration of various oils, including Canola oil, Coconut oil and Banaspati ghee alone and in combination of any two of them simultaneously; while one group served as control was administered normal saline. Baseline body weights of all groups of animals were measured at the start of the study. At the end of 4 weeks of lipid diets, body weight of all groups of animal groups was measured again and their blood was drawn through cardiac puncture for the assessment of TC, LDL-C, HDL, TGs.

Results: Amongst all the groups, Group F (Banaspati ghee + Coconut oil) showed maximum increase in LDL-C, TC,TGs, body weight and decrease in HDL-C (p-value <0.05) in comparison to control.

Conclusion: It is concluded that the combination of Banaspati ghee and Coconut oil may prove to be most effective lipid diet for the induction of dyslipidemia in animal model.

ABBREVIATIONS: TC- Total Cholesterol; LDL-C – Low Density Lipoprotein Cholesterol; HDL-C – High Density Lipoprotein Cholesterol TGs- Triglycerides

Keywords: Animal study, induction of dyslipidemia, wistar albino rats, lipid rich diet

INTRODUCTION

Lipids are the biological molecules that play vital role in many cellular processes in the body; while imbalance of any of the lipid component in the blood predispose patients to a condition known as dyslipidemia **(Wander *et al*., 2017))**. This can occur solely due to inherited factors, but more commonly it is an acquired condition **(Nelson, 2013)**. There is a strong association between altered levels of cholesterol such as increase in LDL-C or decrease in HDL and risk of developing various life threatening disorders including cardiovascular and cerebro-vascular diseases, hypertension, atherosclerosis, coronary heart diseases etc. **(Karr, 2017)**.

Research animals are valuable tools for understanding the pathophysiology of a disease as well as ideal for exploration of new therapeutic interventions for various clinical disorders. In this regard various animal models have been developed successfully to imitate human diseases and are prerequisite for the approval of new drugs by FDA before clinical trials **(Kepplinger, 2015)**. Research animals include mice, rabbits, guinea pigs, rats, sheep, cattle, goats, pigs, cats, dogs, fish, birds, primates, and frogs etc. **(Leong *et al*., 2015)**. Likewise, several researches have been conducted to generate models of dyslipidemia in laboratory animals, in order to understand the relationship between disorders in cholesterol metabolism and atherosclerosis and to test possible treatments for the reduction of circulating cholesterol levels **(Matos *et al*., 2005)**. There are a number of diets that have been employed in the induction of dyslipidemia in animals which include use of various cholesterol rich oils(Such as Canola oil,Soya beanoil, Coconut oil, Banaspati ghee etc.), cholesterol powder and other products enriched in fats and triglycerides **(Yamada *et al*., 2007, Eyres *et al*., 2016)**.

Banaspati ghee is a product of milk, also known as "yellow oil" and is the most frequently consumed dairy product in the subcontinent ,South East Asia **(Hosseini and Asgary, 2012)**. There have been concerns about increased risk of cardiovascular diseases development with ghee consumption since it contains a high percentage of saturated fatty acids including 47.8% saturated fat and 32% Monounsaturated Fatty Acids (MUFA); leading to increased synthesis of cholesterol. In this regard “The American Heart Association” has recommended that the consumption of saturated fats should be less than 7% of energy intake to reduce the risk of cardiovascular diseases **(Eyres *et al*., 2016, Manna *et al*., 2016, Sacks *et al*., 2017)**.

Another widely used edible oil is Coconut oil that hasbeen employed in the food industry for many years; normally termed as Lauric oil/ tropical oil or Confectionery fat. It contains about 90% saturated fats of which majority is Lauric acid (medium chain fatty acids) while 9% are unsaturated fats **(Boateng *et al*., 2016)**. Moreover, Rapeseed oil (well-known as Canola oil) has also emerged as a potential substitute for olive oil since it has a similar monounsaturated fatty acids content to that of olive oil. It has overall beneficial fatty acid profile **(Lauretti and Praticò, 2017)**, owing to the presence of low levels (7%) of saturated fatty acids (SFAs), substantial amounts of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs),such as oleic acid (61%), linoleic acid(21%), alpha-linolenic acid (11%) and tocopherols etc. **(Lin *et al*., 2013)**.

However till date no single diet has turned out to be fruitful in representing the true picture of dyslipidemia in humans, demonstrating derangements in various components of lipids. Therefore this study was conducted with the aim to develop a better animal model of dyslipidemia closely resembling this disorder in humans .We selected Banaspati ghee, Canola oil and Coconut oil alone as well as in combination of two oils at a time to analyze the effects of each diet model on various lipid parameters (HDL, LDL, TGs, Total cholesterol) and body weight of animals.

Ethical approval

The study was approved by Animal Ethics committee Ziauddin University and Protocol No. 2018-005 was allotted.

Standard protocols

All the animals were given twelve-hour light /dark cycle and before start of treatment animals were acclimatized with the environment and were given free access to water. Animals were dealt through all procedures according to CARE guidelines 2010 **(Council, 2010)**.

MATERIALS AND METHODS

Induction of Dyslipidemia

Three edible oils including Canola oil, Coconut oil and Banaspati ghee were given alone and in combination of two oils (according to the groups, mentioned below) in 1:1 ratio at the dose of 10 ml/kg body weight to the albino rats for 28 days (4 weeks).

Grouping of Animals

Animals were randomly selected for grouping. (Total N=42 and 6 animals /group)

Group A: Control group, normal pellet diet with Normal Saline

Group B: Normal pellet diet with Canola oil

Group C: Normal pellet diet with Coconut oil

Group D: Normal pellet diet with Banaspati ghee

Group E: Normal pellet diet with Coconut oil + Canola oil

Group F: Normal pellet diet with Coconut oil + Banaspati ghee

Group G: Normal pellet diet with Banaspati ghee + Canola oil

Experiment

This Experimental animal study was conducted at the animal house of Faculty of Pharmacy Ziauddin University Karachi, while laboratory work was performed at Ziauddin University (MDRL-1). For this study 9 weeks old, 42 male Albino Wistar rats, weighing 300-400g were purchased from Liaquat National University and Hospital. After adapting with the environment, animals were randomly assigned into seven groups (n=6/group) and were maintained on a standard animal diet purchased from local supplier in sufficient quantity for the entire period (4 weeks) of the study and Water adlibitum. The three chosen edible oils include Canola oil, Coconut oil and Banaspati ghee were administered at the dose of 10ml/kg body weight to all the groups (except group A which is the control group) with feeding tube according to the protocol. Body weight of all the animals was measured at baseline using the standard weight machine.

At 28thday (4 weeks) of administration of respective oils (according to group) all animals were anesthetized by injecting ketamine at the dose of 50mg/kg. **5** ml blood was drawn via cardiac puncture of all the rats in EDTA containing vacutainer tubes, and was transferred to MDRL-1 for the analysis of lipid profile and Liver function tests (LFTs).After centrifugation of blood ,serum was separated and the levels of Total cholesterol (make:human packing kit), Triglycerides(make: human packing), LDL-c (make:diasys packing) , HDL cholesterol (make:human precipitation)and were assessed by automated analyser for which Diasys packing and Human packing kits were usedin order to evaluate the outcome of studied diets on aforementioned parameters.

STATISTICAL ANALYSIS

Data entry and analysis were conducted on SPSS version 20. Anova followed by Post Hoc Tukey's test was applied for inter and intra group comparison of various hematological parameters. P value less than 0.05 was considered as significant.

RESULTS

Table 1 is representing significant (p-value=0.000) increase in all the lipid parametersalong with a significant (p-value=0.000) decrease in HDL in groups F (Coconut oil = Banaspati ghee),G (Banaspati ghee + canola oil) and D (Banaspati ghee alone) as compared to Group A (control group). While Group C (coconut oil alone) displayed significant decrease in all the lipid parameters significantly increased HDL as compared to other groups. Whereas group B (canola oil alone) showed significant decrease in all lipid parameters when compared to controls.

Table 1: Mean± SD of Various Lipid parameters and Body weights of animals (N=42)

Hematological Parameter	Group A Control n=6	Group B (CO.A) n=6	Group C (COC.A) n=6	GroupD (BG.A) n=6	Group E (COC+ CO) n=6	Group F (COC+BG) n=6	Group G (CO+ BG) n=6	P value
TC	100.50 ±0.548	88.67 ±1.033	97.33 ±0.816	122.33 ±0.816	92.50 ±1.517	126.17 ±1.472	121.50 ±1.049	0.000*
LDL	77.00 ±0.632	64.67 ±0.816	74.00 ±0.632	102.00 ±1.265	72.83 ±1.169	105.67 ±1.366	102.17 ±0.753	0.000*
HDL	35.50 ±0.548	29.83 ±0.753	39.83 ±1.169	20.00 ±0.632	33.17 ±0.983	19.67 ±1.506	22.17 ±1.472	0.000*
TGs	79.50 ±0.548	76.50 ±1.049	75.83 ±1.472	110.67 ±2.733	74.17 ±1.472	114.83 ±0.983	90.90 ±17.194	0.000*
Body wt.	392.67 ±2.944	384.17 ±0.983	394.50 ±2.429	408.17 ±0.408	386.83 ±0.753	416.17 ±1.329	405.83 ±1.169	0.000*

*p value<0.05 = significant CO = Canola oil COC = Coconut oil BG = Banaspati ghee (A = alone)

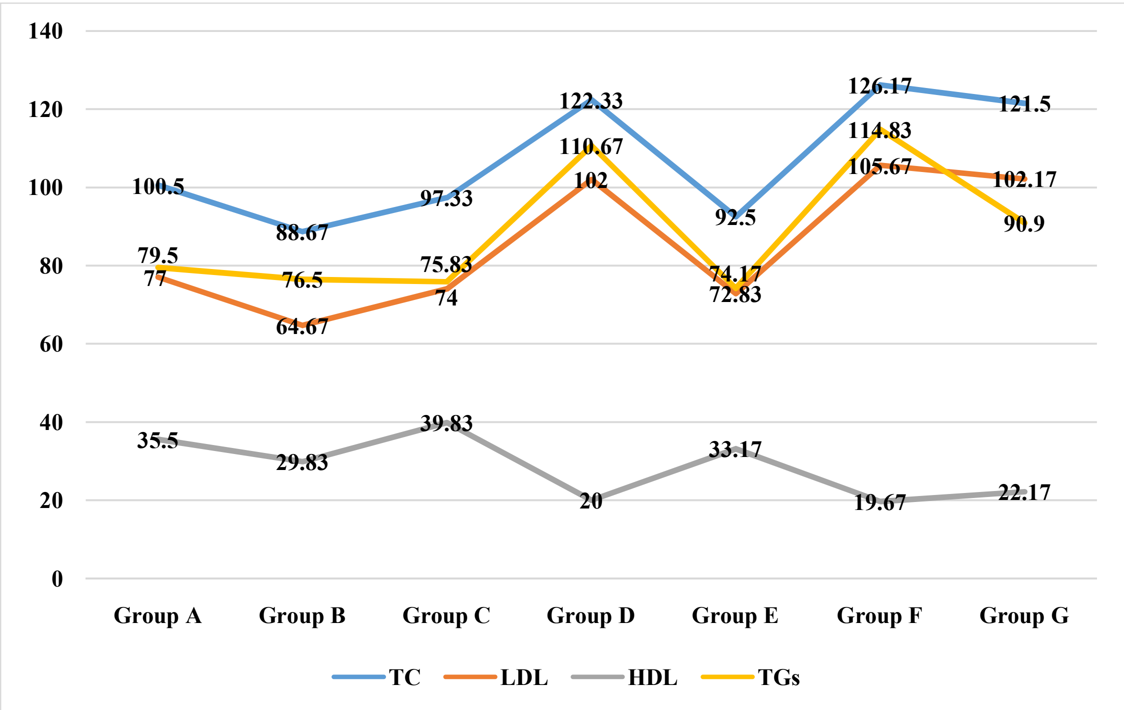

Figure 1: Comparison ofmean lipid parameters of various diet groups

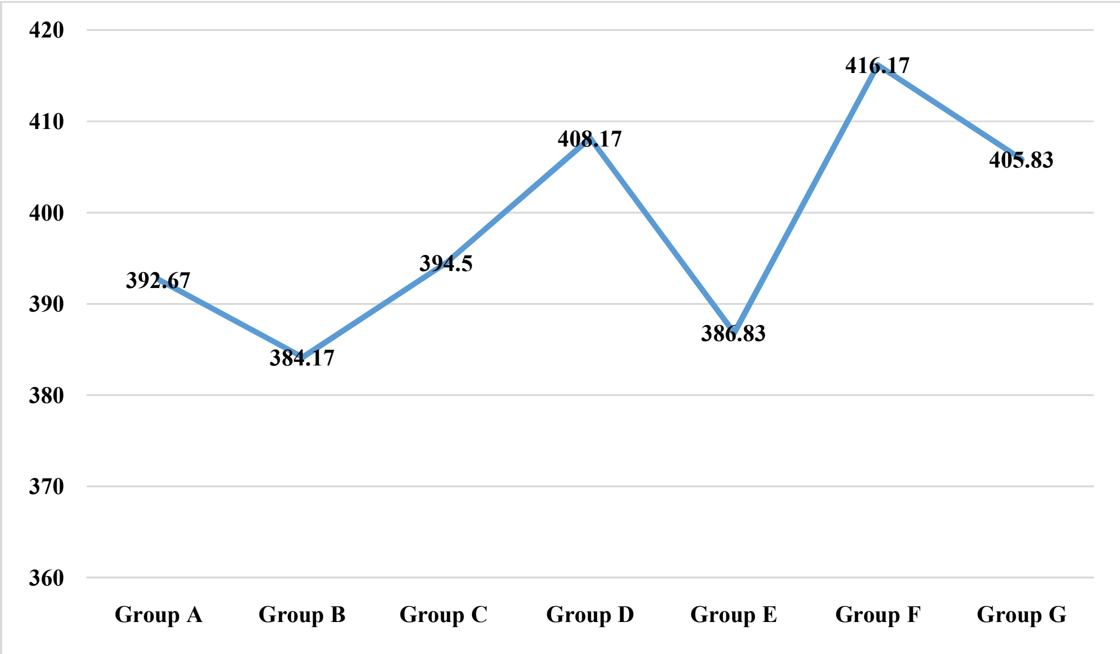

Figure 2: Comparison of mean body weights of various diet groups

Post hoc analysis displayed that except for Groups D and G there were significant ($p<0.05$) differences in total cholesterol among all groups. While groups' comparisons for LDL displayed significant p-value for all excluding the comparison of Group C with group E and group D with group G, showing non-significant p-values, 0.409and 1.000 respectively. When HDL was compared there was a significant difference amongst all the groups, but group D with group F have insignificant p-value (0.998). However the comparison of triglycerides between all the groups found to be significant among groups A and group B (p-value 0.077), group B and group C (p-value 0.994), group B and group E (p-value 0.280), group C and group E (p-value 0.664). As far as body weight is concerned, after post hoc analysis we found out that there was significant difference between all the groups (p--value <0.05) excluding controls, Group A and group C (p=value 0.489), group B and group E (p-value 0.110), group D and group G (p-value 0.218) . After all the comparisons most significant decrease in all the lipid parameters was shown in Group F (B.G+COC.O).

DISCUSSION

Currently several fatty diets have been evaluated for the development of animal models of dyslipidemia, displaying variable effects on lipid levels; since dyslipidemia is a heterogeneous disorder, characterized by derangements either in various components simultaneously or in a single component such as isolated increase in LDL, TC, LDL and

TGs. Therefore specific animal model of dyslipidemia according to nature of the study has to be generated in order to obtain accurate results **(Yamada *et al*., 2007, Karimi, 2012)**. Therefore in our research we assessed various lipid enriched diets alone and in combination for the induction of dyslipidemia with altered lipid parameters representing a wide range of these disorders.

The diet containing the combination of Banaspati Ghee and Coconut oil (group F)was found to be far superior in increasing all the lipid parameters significantly with decrease in HDL **(Table 1)**. No comparative data is available for this combination ,however the possible explanation for our results is that both oils are rich in TFA (Trans-Saturated Fatty acids) and SFA (saturated fatty acids) and various animal studies have shown that saturated fats increase LDL cholesterol by inhibiting LDL receptor activity and enhancing apolipoprotein (apo) B containing lipoprotein production **(Takeuchi and Sugano, 2017)**. It was also revealed that there is a direct relation between dietary content of saturated fatty acids and LDL cholesterol levels as saturated fats increase in diet the later increase accordingly **(Siri-Tarino *et al*., 2010, Mohammadifard *et al*., 2013)**. Our results for the Banaspati ghee alone also showed major increase in LDL-C, TC, TGs while decreasing HDL. Our results are similar to a study conducted by **Alam Zeb *et al*** which displayed a significant increase in all serum lipid levels in rabbits after ingestion of Banaspati ghee alone in oxidized and unoxidized forms **(Zeb and Uddin, 2017)**. Our study and other similar studies have proved the role of saturated fats in increasing LDL cholesterol as Banaspati ghee comprises more than 20% of Trans fatty acids while 60% are Saturated fatty acids **(Dhaka *et al*., 2011)**. However contrary to our study results, **Kumar *et al*** displayed that the diets enriched in Banaspati ghee cause serum cholesterol to decrease and did not increase the risk of CHD as compared to mustard oil in urban population of India **(Manna *et al*., 2016)**.These contrasting results could be due to selection of different subjects and presence of various factors such as consumption of fiber, physical activities, hormonal ,genetic and socioeconomic factors that could confound studies in human being.

Our data displayed that the diets containing canola oil alone and in combination with other oils showed significant effects on lipid parameters including decrease in LDL,TC and TGS, whereas HDL remained unaffected **(Figure 1)**. These effects of Canola oil could be attributed to its high oleic acid content , low saturated fat and presence of omega-6 and omega-3 fatty acids in a ratio 2:1, eventually helps to reduce LDL-C and total cholesterol levels **(Saedi *et al.*, 2017)**. Our results are similar to a number of studies including meta-analysis done by **Lukas Schwingshackl *et al.* (Schwingshackl *et al.*, 2018)**.

Regarding Coconut oil, our results have shown significant increase in HDL cholesterol as compared to other groups **(Figure 1)**, similar to various studies including Bianca de Oliveira et al., assessed effects of diet enriched in coconut oil on Wistar rats and found significant increase in HDL **(Schumacher *et al.*, 2016)**. Likewise, **Chinwong *et al* in 2017** also reported raised HDL levels in all the subjects. The most striking reason behind increase in HDL by coconut oil is its high proportion of medium chain fatty acids, Lauric acid (C12:0) and Myristic acid (C14:0) **(Chinwong *et al.*, 2017)**, that are rapidly absorbed from body and are abundantly present in coconut oil **(Khaw *et al.*, 2018)**. They both tend to increase the production of apolipoprotein A1 which eventually increase HDL-C1 production **(Mooradian *et al.*, 2005)**. However a study conducted by **Hari Sharma *et al*** stated that coconut oil shows dose dependent decrease in LDL-c, TC and TGs when compared to groundnut oil in Sprague-Dawley outbred rats and the justification of the result says that Dietary ghee have no effect on a key enzyme of cholesterol synthetic pathway i.e HMG CoA reductase activity in liver microsomes and hence increased the excretion of bile constituents and lowered serum cholesterol levels **(Sharma *et al.*, 2010)**.

Presently Obesity has been well-thought-out as a global endemic disease, is a component of metabolic syndrome and usually accompanied by atherogenic dyslipidemia and other health threats **(Klop *et al.*, 2013)**. As shown in **Figure 2**, there is significant increase in body weight in the group kept on Banaspati ghee alone and in combination with coconut and canola oils. These results are parallel to the reports of **Vandana Dhaka *et al*** that also showed significant increase in body weight of animals after ingesting Banaspati ghee asit possess huge fraction of Trans Saturated fatty acids **(Dhaka *et al.*, 2011)**. No parallel or comparative study available for the combination of Banaspati ghee with coconut or canola oil. However some studies have reported contrasting results when Banaspati ghee was compared with other oils such as **Yogita Surendra *et al*** showed significant decrease in body weight in Wistar rats taking ghee as compared to butter **(Karandikar *et al.*, 2016)**. Our study also displayed a significant decrease in weight in the groups givencanola oil alone and combination of canola and coconut oils. Several studies have documented equivalent results, **Heitor O. *et al*** in the year 2019, there is decrease in body weight of 20 overweight patients with the use of 30 mg capsule of coconut oil and hypocaloric diet **(Santos *et al.*, 2019)**. Accordingly for canola oil various studies report similar results such as study conducted by **Xiaoran Liu *et al.*** showed significant decrease in body weight in the participants consuming canola oil when compared with flax seeds and sunflower oils **(Liu *et al.*, 2016)**.The explanation for weight loss is quite difficult as this is a very complicated process dependent up on multiple factors including diet, life style, neuronal and hormonal circuits, metabolic functional capacities and above all genetic makeup. Nevertheless canola and coconut oil are recently identified as healthy oils and are a part of certain weight reducing diets such as keto and Atkins diets etc. **(Melo *et al.*, 2018)**.

CONCLUSION

It is concluded that the diets rich in Banaspati ghee and coconut oil in a combination have profound effects on all lipid parameters especially increase in LDL-C along with decrease in HDL cholesterol, hence can induce dyslipidemia in animal in a short span of time i.e within 28 days. Furthermore this model of dyslipidemia may also be suitable to analyze various lipid parameters simultaneously and could be promising to study new interventions having wide range of effects on all lipid parameters.

Whereas diets rich in canola oil alone and coconut oil alone both can help in reducing lipid parameters making it a healthy substitute to decrease the risk of dyslipidemia and related co-morbids.

Limitations

In our study the major limitation was that the animals were observed only for 28 days rather than observing and following the same animal on various point of the time during the study period. Furthermore, we could not work on other methods of inducing dyslipidemia in our study animals or in other animal species.

Strengths

To the best of our knowledge this is the first study in which various diet combinations have been tested on Wistar rats and has been evaluated the best possible diet to develop model of dyslipidemia as real as possible.

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Ethical Approval: Animal ethics committee of Ziauddin University approved the study.

Patients consent form: Not applicable.

Authors Contribution: The concept of study, data analysis, drafting, and finalizing of the results were done by Dr. Nisha Zahid. The article was critically reviewed and finally drafted by Dr Shehla Shaheen. Finally reviewed and approved by Dr Zahida Memon. Laboratory/instrument based work was performed with the help of Dr. Shehzano, Dr. Dabeeran and Dr. Akhtar Ali.

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