



The Effect of Ginger (*Zingiber officinale*) on Diet Induced Hyperlipidemia and Tissue Histology in Adult Female Wistar Rats: A Biochemical and Histopathological Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author ORA carried out the biochemical analysis and experimental animal study. Author IAK performed the histologic study. Author AAI designed the study and wrote the draft. All authors read and approved the final manuscript.

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ABSTRACT

The study is aimed at determining the atheroprotective property of ginger (*Zingiber officinale*) aqueous extract to rats fed with a high-fat diet. Each group consisted of 5 rats. Group 1 represent the control group and received normal rat pellets; groups 2-5 were maintained on high-fat diet for 6 weeks to establish hyperlipidemia. Groups 1, 2, 3, 4 and 5 received distilled water, distilled water, 400, 500 and 600 mg/kg (body weight) *Zingiber officinale* respectively for 4 weeks. Serum concentrations of triglycerides (TG), total cholesterol (TC) and high-density lipoprotein-cholesterol (HDL-C) were determined using standard biochemical methods. Histologic examination of tissues

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was by Hematoxylin-Eosin staining technique. Data were analyzed using Student's t-test and $P < 0.05$ was considered significant. A decrease in the mean concentrations of TG, TC, low-density lipoprotein (LDL-C) and increase in HDL-C concentration of each of the treatment groups (3, 4, 5) were observed when compared with that of group 2. In rats fed high-fat diet, histologic abnormalities were observed for the heart, liver, and kidney which were not adequately corrected by ginger treatment. Results suggest that at dosage levels studied, aqueous ginger extract ameliorated biochemical but not histologic consequences of the high-fat diet.

Keywords: *Ginger extract; lipid profile; high-fat diet; tissue histology.*

1. INTRODUCTION

The high-fat diet has been recognized as a cause of derailment in the histoarchitecture of many tissues in men and different types of experimental animals. Most notable of the organs affected by hyperlipidemia is the heart. Cardiovascular diseases continue to be the leading cause of death globally [1]. Coronary heart diseases reported to be the fifth leading cause of deaths in the year 1990 by the World Health Organization (WHO), are estimated to top the list by the year 2020. When high-fat diets induced cardiac abnormalities (e.g. hypertension/coronary heart disease) are left unattended, they can negatively impact other organs such as the kidney. Moreover, nonalcoholic fatty liver disease (NAFLD) has been reported to occur from the consumption of a high-fat diet [2,3].

It is now well established that elevated levels of cholesterol, triglycerides, Low-Density Lipoprotein cholesterol (LDL cholesterol), Very Low-Density Lipoprotein cholesterol (VLDL cholesterol) and decreased levels of High-Density Lipoprotein cholesterol (HDL cholesterol) are closely associated with coronary heart diseases and atherosclerosis [4,5]. These are indices that are also well known to be linked with the high-fat diet. According to Akhiani, et al. [6] and Nnami, et al. [7] clinical complications of atherosclerosis could be diminished and life prolonged if plasma lipid levels are lowered. It has also been found that long-term, effective treatment of hyperlipidemia can decrease the occurrence of ischemic heart disease [8]. But many promising agents developed have serious side effects, especially on adrenal function.

The medicinal plants have been used in traditional medicine for millennia, so it is not a recent development [9]. Today more than 80% of the populations in the developing countries rely mainly or partially on medicinal plant for health care purposes [10]. Of the top 150 proprietary

drugs used in the United States of America (USA), 57% contain at least one major active compound currently or once derived from plants [11]. This illustrates the relative importance of plants in traditional healthcare. A medicinal plant has been defined as any plant which in one or more of its organs contains a substance that can be used for therapeutic or preventive purposes or which are precursors for the synthesis of useful drugs [12,13]. Many medicinal plants have been used in various traditional systems, for lipid management.

Zingiber officinale has antioxidant properties and data are available that suggest its importance in the management of many disease conditions. The objective of the study is to investigate the effect of aqueous *Zingiber officinale* extract on high-fat diet-induced hyperlipidemia and histology of heart, liver and kidney. The study involves the use of aqueous extract rather than high hydrostatic pressure or ethanolic extract, this is because most Nigerians that use ginger for therapeutic purposes use aqueous rather than ethanolic extract.

2. MATERIALS AND METHODS

The animal study was carried out in conformity with national and international laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research; as promulgated and adopted by United States Institutes of Health (1985). Twenty-five female Wistar rats weighing between 100-125 g were randomly divided into 5 groups of 5 animals. Animals were kept in the Animal House at Mercy-land, Ladoke Akintola University of Technology, Osogbo. Rats in the first group (control) were fed normal pellets throughout the duration of the experiment. Rats in the 2nd, 3rd, 4th and 5th groups were fed high-fat diet for 6 weeks to induce hyperlipidemia. Hyperlipidemia was experimentally induced by incorporating dried animal fat (5 g), egg yolk (20 g) and coconut oil (20 mL) to every 100 g of rat chow daily. Hyperlipidemia was confirmed

biochemically before administration of aqueous extract of *Zingiber officinale* commenced. Rats in groups 3, 4 & 5 were administered via gastric gavage with varying concentrations of *Zingiber officinale* of 400, 500 and 600 mg/kg body weight respectively for another four weeks. All of the rats were given unrestricted access to feed and clean water throughout the experiment. Meanwhile, for the same period of 4 weeks, rats in groups 1 and 2 were administered with distilled water.

2.1 Preparation of Ginger Extract

Ginger (*Zingiber officinale*) was purchased from a local market. It was washed, dried and milled to a powdered form, after which it was made into aqueous form. The concentrations of *Zingiber officinale* were prepared per body weights of rats in the test treatment groups (400 mg/kg, 500 mg/kg and 600 mg/kg BW).

2.2 Sample Collection and Biochemical Analysis of Lipid Profile

After cervical dislocation, fasting blood samples were collected by cardiac puncture into plain sample bottles, centrifuged at 2500 rpm for 15 minutes and stored at -20°C prior to analysis. Estimation of total cholesterol and triglycerides were by the methods of Allain, et al. [14] and Bucollo and David [15] respectively. Plasma LDL – cholesterol was calculated using Friedewald’s formula.

Histology: Organs were fixed with neutral formalin 10%. The tissues were dehydrated

through ascending grades of alcohol. Thereafter, it was cleared in xylene and finally embedded in paraffin wax. Using a rotary microtome, specimens were sectioned at 4 µm. They were stained with Hematoxylin and Eosin staining procedures. Photomicrographs were taken and magnification was at X 400.

3. RESULTS

The results of different fractions of lipid profile estimated are summarized in Fig. 1. Significant increases ($p < 0.05$) in the mean values for total cholesterol, triglycerides, and LDL-C but significant decreases ($p < 0.05$) in levels of HDL-C of rats fed high-fat diet (group 2) were observed compared with control- group 1 (Table 1). On the other hand, there were no significant differences ($p > 0.05$) in the mean total cholesterol, triglyceride, LDL-C, and HDL-C in those rats treated with varying concentrations (groups 3, 4, 5) of *Zingiber officinale* when compared with those in control- group 1 (Table 1). This study also revealed significant decreases ($p < 0.05$) in the mean values of TC, TG, and LDL-C, but significant increases ($p < 0.05$) in HDL-C concentrations in Wistar rats treated with varying concentrations of *Zingiber officinale* (400, 500, 600 mg/kg) when compared with those in group 2 (untreated high-fat diet group) as shown in Table 2.

Fig. 2 showed the histology results of sections of the heart in which there was a normal presentation for control but increased lipid droplet accumulation infiltrating the elastic walls as well as thickening of the elastic connective tissue in the high-fat group (group 2).

Table 1. Comparison of Mean±SD of lipid profile of rats in groups 1-5

Parameters/ Groups:	TC Mean±SD	TG Mean±SD	HDL-cholesterol Mean±SD	LDL-cholesterol Mean±SD
Group 1	3.05±0.10	1.13±0.22	1.18±0.13	3.75±0.10
Group 2	5.06±0.13	1.98±0.17	0.65±0.27	4.08±0.46
t value	24.211	6.074	3.584	4.512
p-value	0.000	0.001	0.021	0.017
Group 3	3.15±0.19	0.95±0.33	0.78±0.17	3.43±0.32
t value	0.926	0.877	3.771	1.938
p-value	0.390	0.414	0.070	0.133
Group 4	3.05±0.10	1.23±0.09	1.38±0.08	3.85±0.09
t value	0.000	0.828	2.242	0.361
p-value	1.000	0.439	0.417	0.730
Group 5	3.35±0.43	0.95±0.37	1.20±0.08	3.88±0.09
t value	1.342	0.812	0.333	1.806
p-value	0.228	0.448	0.750	0.121

Abbreviations: SD- standard deviation; TC- total cholesterol; TG - triglycerides; HDL - chol- high density lipoprotein= chol; LDL-cholesterol- low density lipoprotein-cholesterol. *P < 0.05 is considered significant

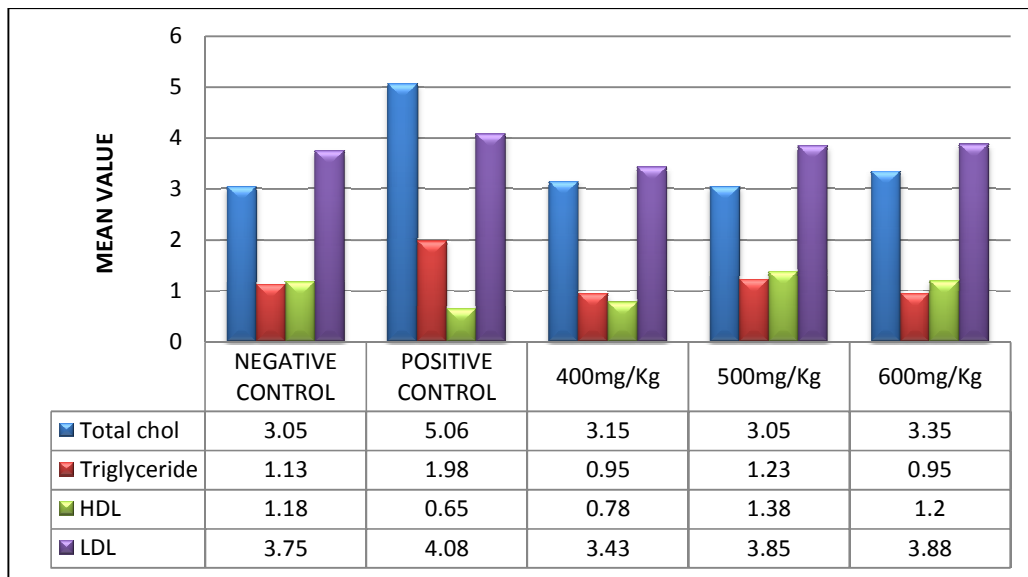


Fig. 1. Summary (graphically) of serum lipid profile levels of the 5 groups of experimental rats. In each case, the results are expressed in mmol/L

Rat in groups 3, 4 and 5 manifested various degrees of histologic changes. Group 3 (myocardial tissue with increased mononuclear cell infiltration and thickening of the elastic connective tissue); group 4 (myocardial tissue with normal cytoarchitecture and thickening of the elastic connective tissue); and group 5 (myocardial tissue with regenerating myocardial cytoarchitecture and mild hemorrhage within elastic tissue). Histology results of hepatic tissue in Fig. 3 revealed normal cytoarchitecture and uniformly radiating sinusoids (control); increased vacuolation indicating lipid droplet accumulation (group 2); and various changes for groups 3 (vacuolation in

the sinusoids and lipid droplet accumulation); 4 (lipid vacuolation within the sinusoids and mononuclear cell infiltration and hemorrhage); and 5 (regeneration of hepatocytes with resolving vacuolation and mild hemorrhage). Fig. 4 revealed the histology results of sections of kidney in which normal architecture was revealed for group 1; moderately distorted cytoarchitecture and accumulation of lipid droplets in the lumen (group 2); moderately distorted cytoarchitecture (group 3); and for groups 4 (the glomeruli appear normal, with moderate interstitial congestion and hemorrhage) and 5 (the glomeruli appear normal with mild cortical congestion).

Table 2. Comparison of Mean±SD of lipid profile of untreated and treated (with different doses of *Zingiber officinale*) rats fed high-fat diet

Parameters/ Groups:	TC Mean±SD	TG Mean±SD	HDL- chol Mean±SD	LDL- chol Mean±SD
Group 2	5.06±0.13	1.98±0.17	0.65±0.27	4.08±0.46
Group 3	3.15±0.19	0.95±0.33	0.83±0.26	3.43±0.25
t value	16.380	5.495	0.938	5.301
p-value	0.000	0.002	0.024	0.002
Group 4	3.05±0.10	1.23±0.09	1.38±0.13	3.85±0.25
t value	24.211	7.661	4.973	3.657
p-value	0.000	0.000	0.003	0.011
Group 5	3.35±0.44	0.95±0.37	1.20±0.08	3.88±0.09
t value	7.487	5.034	3.973	3.982
p-value	0.003	0.002	0.007	0.003

Abbreviations: SD- standard deviation; TC- total cholesterol; TG- triglycerides; HDL-*chol*- high density lipoprotein= *chol*; LDL-*chol*- low density lipoprotein-*chol*. *P <0.05 is considered significant

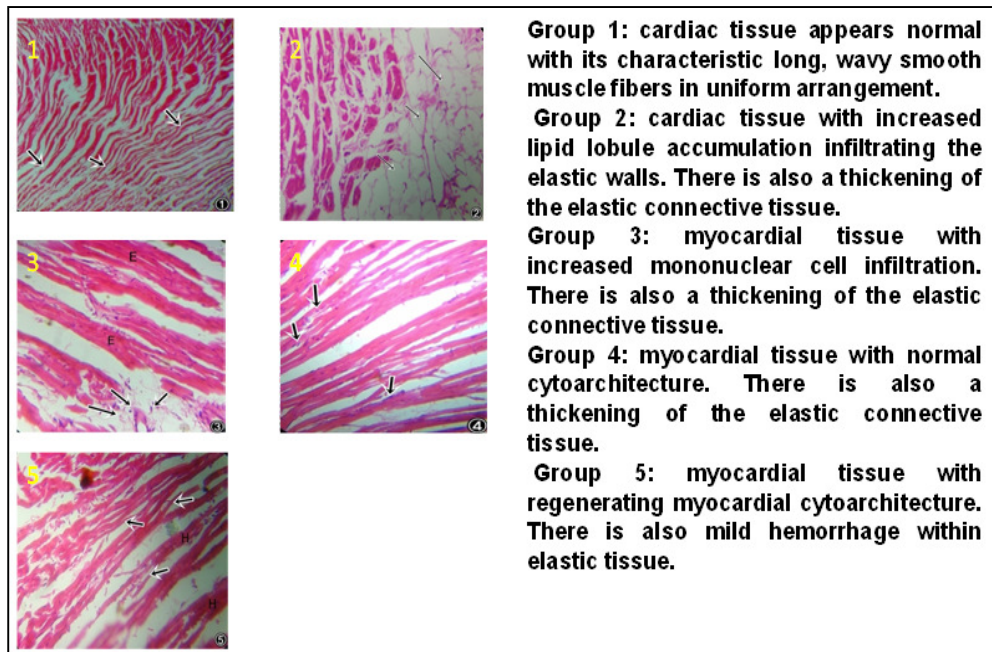


Fig. 2. Photomicrographs of hearts of rats fed high-fat diet and treated with different doses of aqueous ginger

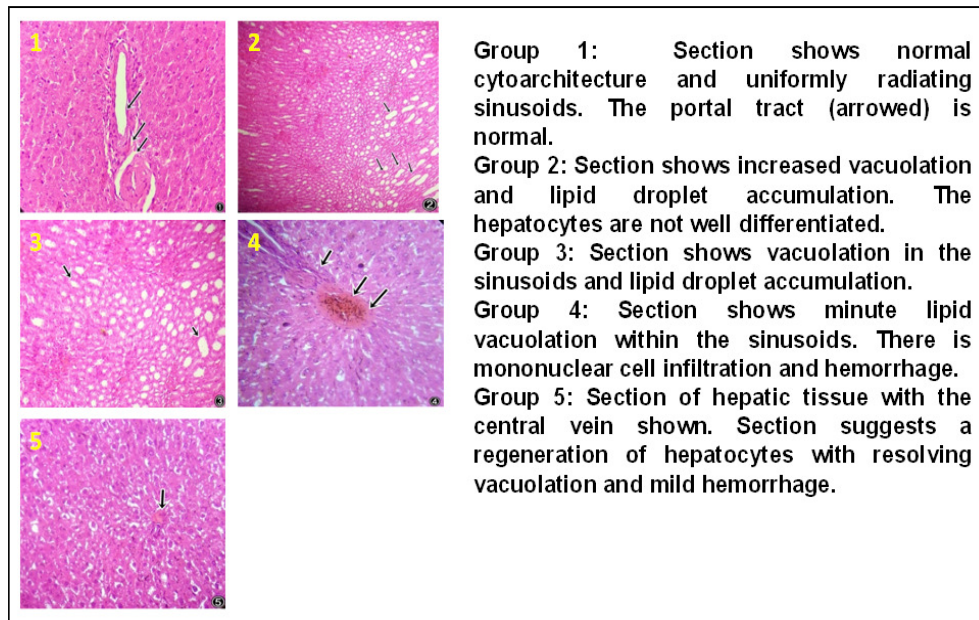


Fig. 3. Photomicrographs of livers of rats fed high-fat diet and treated with different doses of aqueous ginger

4. DISCUSSION

The three components (animal fat, egg yolk, coconut oil) that were compounded to induce hyperlipidemia in the experimental rats are those

food sources commonly consumed by humans and which can serve as indicators of what may obtain in some other mammalian species that consume them. The rats in the high-fat diet group had significantly high levels of cholesterol,

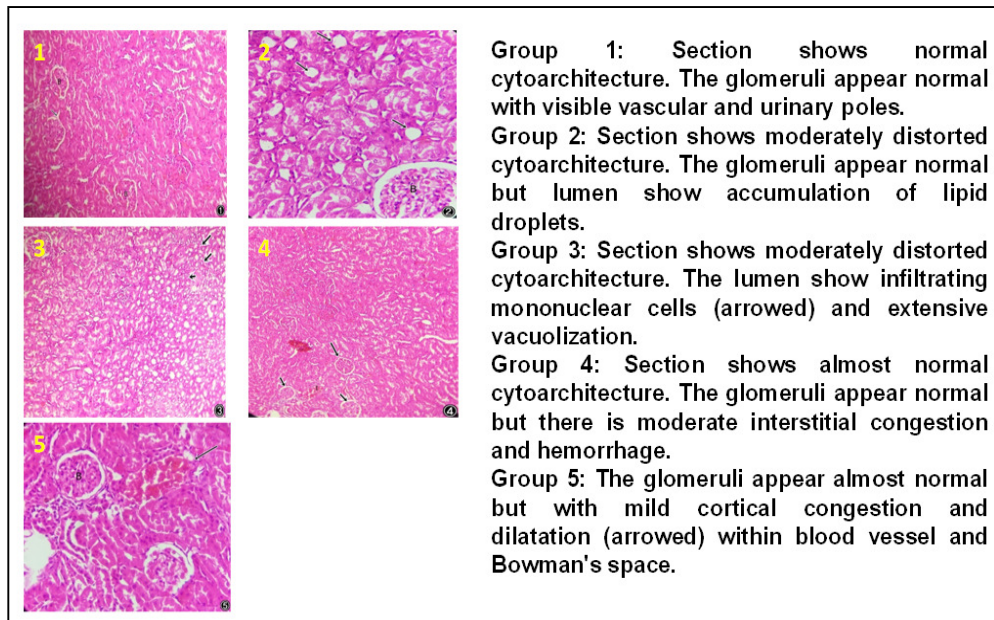


Fig. 4. Photomicrographs of kidneys of rats fed high-fat diet and treated with different doses of aqueous ginger

triglycerides, Low-Density Lipoprotein cholesterol (LDL cholesterol), and decreased levels of High-Density Lipoprotein cholesterol (HDL cholesterol) compared with control (Table 1). This portrays that rats fed high-fat diet may be susceptible to increase risk in coronary heart diseases and atherosclerosis [4,5]. Giving credence to this is the histology result that showed that cardiac tissue had increased lipid droplet accumulation as well as thickening of the elastic connective tissue. The diet caused abnormal histoarchitecture of not only the heart but also of the liver and kidney as shown in Figs. 2, 3, 4. The result is in agreement with the observation of Altunkaynak et al. [16]. According to them, the high-fat diet resulted not only in obesity but also in renal deformities due to histological changes such as dilation, tubular defects, inflammation, and connective tissue enlargement of the kidney. Hassan et al. [17] also observed that fatty liver was induced in a group of rats that were fed high-fat diet for 6 weeks, with histologic consequences such as disturbed hepatic architecture, dilation and congestion of central veins, blood sinusoids and portal veins. The slight difference in histology results of both studies (17; and the present study) may be due to differences in components of the high-fat diet of each group. While Hassan et al. [17] prepared high-fat diet by incorporating 20% animal fat + 1% cholesterol to the standard diet; for the present study high-fat diet was made by

incorporating dried animal fat (5 g), egg yolk (20 g) and coconut oil (20 mL) to every 100 g of rat chow.

The significant decrease in the levels of LDL of rats treated with various doses of the extract compared with high-fat diet group is an indication that aqueous extract of ginger may possess protective effects on biochemical markers of hyperlipidemia. Elevated LDL has been recognized to be a hidden risk factor for cardiovascular diseases; it also enhances atheroma (plaque) formation in arteries. In addition, rats in different treatment groups also manifested a significant increase in the levels of HDL compared with the high-fat diet group. There are various reports that suggest that a high HDL level is protective against coronary heart diseases. The protective effects of ginger in ameliorating markers of hyperlipemia can be ascribed to radical scavenging antioxidant polyphenols found in ginger [18]. Aside from polyphenols flavinoids are also present in ginger. This basic mechanism has been reported to enhance the process of regeneration through the elimination of free radicals, supplying an alternative substrate for unsaturated lipids in the membrane as well as encouraging the repair mechanism of the damaged cell membrane when rats fed high-fat diet were treated with pomegranate [17].

Two other indices that enhance hyperlipidemia related events like cardiovascular diseases i.e. total cholesterol and triglyceride were significantly increased in rats in the untreated high-fat group compared with control rats and significantly lower in all ginger extract treatment groups compared with the untreated high-fat group. Results of serum lipid profile seem to support the protective role of this extract in all ginger treatment groups as summarized in Fig. 1 but the histology results of sections of heart and liver still revealed a slight abnormality in tissue architecture. This may be an indication that the dosage levels 400, 500, 600 mg/kg are not adequate to sufficiently ameliorate the histologic consequences of high-fat diet-fed. Kim, et al [19] who observed histologic amelioration of ginger treatment used a dose of 800 mg/kg rather than the highest dose of 600 mg/kg used for the present study.

Some past studies have shown that ginger ameliorates not only biochemical markers of hyperlipidemia but histologic ones as well. Differences in result outcomes may be linked not only to differences in dosage levels employed but the type of extract used as well. Kim, et al. [19] used both hot-water extract of ginger (WEG) as well as high-hydrostatic pressure extract (HPG); according to them, HPG featured not only serum and hepatic lipid levels which were lower than HFD group but also for WEG and HPG groups, mRNA levels of adipogenic genes were lower compared with HFD group. But only HPG had a lower mRNA level of pro-inflammatory cytokines than HPG group. They opined that all these may however be linked with differences in polyphenols or flavonoids in ginger extracted using different methods. They reported that compared with steam extracted ginger, ethanolic ginger extract exerted 4-fold and 30-fold higher levels of polyphenols and flavonoids (Kim, et al. 2019). There seem to be differences in organ susceptibility to ginger-extract exposure as the renal cells of rats fed high-fat diet and treated with ginger extract showed almost normal cytoarchitecture for 500 mg/kg and 600 mg/kg groups. Furthermore, the 4-week period of extract treatment may be insufficient going by the histology results of both the liver and heart sections.

Since clinical complications of atherosclerosis could be diminished and life prolonged if plasma lipid levels are lowered, a more effective way of using the ginger extract in the management of biochemical and histologic consequences of a

high-fat diet may serve as preventive means for heart-related diseases as well as have an impact in the occurrence of ischemic heart disease. For many Nigerians who have adopted ginger for treatment of one disease or the other by soaking this rhizome roots in water for several hours and consuming the extract, this study indicates that such extraction method may not be ideal.

5. CONCLUSION

Compared to other herbs used in the treatment of cardiovascular diseases, ginger has an edge or advantage over many other alternative drugs because, in most communities, ginger is an integral part of the diet. This means that it can be easily incorporated into day to day food preparation. And based on the aforementioned serum lipid profile results, it may be speculated that ginger is an effective herbal remedy for a risk factor of IHD i.e. hyperlipidemia. But when this is considered in association with histologic results, it seems as if the ability of ginger in its aqueous extract form to correct the overall negative impact of a high-fat diet is limited.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nowbar AN, Gitto M, Howard JP, Francis DP, Al-Lamee R. Mortality from ischemic heart disease Analysis of data from World Heart Organization and coronary artery disease risk factors from NCD risk factor collaboration. *Circulation: Cardiovascular Quality and Outcomes*. 2019;12:e005375.
2. Kakimoto PA, Kowaltowski AJ. Effects of high fat diets on rodent liver bioenergetics and oxidative imbalance. *Redox Biology*. 2016;8:216-225.
3. Velazquez KT, Bader JE, Sougiannis AT, Carson MS, Chatzistamou I, Carson JA, Nagarkatti M, Murphy EA. Prolonged high-

- fat-diet feeding promotes non-alcoholic fatty liver and alters gut microbiota in mice. *World Journal of Hepatology*. 2019;11(8): 619-637.
4. Omran MOA., Al-Mayahb JY, Al-Mousawib NR. Al-Aoboodib AGH. Effects of garlic on haemostatic parameters and lipid profile in hyperlipidemia rats: antiatherogenic and antithrombotic effects. *Eastern Journal of Medicines*. 2006;11:13-18.
 5. Sanjay KB, Maulik S. Effect of garlic on cardiovascular disorders. *Nutrition Journal*. 2002;1: 4-30.
 6. Akhani, SP, Vishwakarma SL, Goyal RK. Anti-diabetic activity of *Zingiber officinale* in streptozotocin-induced type I diabetic rats. *Journal of Pharmacy and Pharmacology*. 2004;56(1):101–115.
 7. Nammi S, Sreemantula B, Roufogalis D. Protective effects of ethanolic extract of *Zingiber officinale* rhizome on the development of metabolic syndrome in high-fat diet-fed rats. *Basic and Clinical Pharmacology and Toxicology*. 2009; 104(5):366–373.
 8. Zoda D, Giammona R, Schifilliti S. Treatment strategy for dyslipidemia in cardiovascular disease prevention: Focus on old and new drugs. *Pharmacy: Journal of Pharmacy Education and Practice*. 2018;6(1):10. DOI: 10.3390/pharmacy6010010
 9. Abu-Rabia A. Urinary diseases and ethnobotany among pastoral nomads in the Middle East. *Journal of Ethnobiological and Ethnomedicine*. 2005; 1:4-4.
 10. Setzer MC, Werka JS, Irvine AK, Jackes BR, Setzer WN. Biological activity of rainforest plant extracts from far north Queensland, Australia. In: Williams LAD ed. *Biologically Active Natural Products for the 21st Century*. Research Signpost, Trivandrum 695 023, Kerala, India. 2006; 21–46.
 11. Grifo F, Rosenthal J. *Biodiversity and human health*. Island Press, Washington DC, USA; 1997.
 12. WHO/AFRO, author. *African Traditional medicine' brazzaville: Technical report series*. Report of the regional expert committee. 1976;1:3-4.
 13. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med*. 2013; 10(5):210-219.
 14. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem*. 1974; 20:470-5.
 15. Bucollo G, David M. Quantitative determination of serum triglycerides by use of enzyme. *Clin Chem*. 1973;19:476-482.
 16. Altunkaynak ME, Ozbek E, Altunkaynak BZ, Can I, Unal D, Unal B. *J Anat*. 2008;212(6):845-852.
 17. Hassan NF, Soliman GM, Okasha EF, Shalaby AM. Histological, immune histochemical and biochemical study of experimentally induced fatty liver in adult male albino rat and the possible protective role of pomegranate. *Journal of Microscopy and Ultrastructure*. 2018;6(1): 44-55.
 18. Kim S, Lee M, Jung S, Sun H, Park S, Kang B, Kim S, Kim I, Kim C, Kim Y. Ginger extract ameliorates obesity and inflammation via regulating microRNA-21/132 expression and AMPK activation in white adipose tissue. *Nutrients*. 2018;10 (11):1567. Available:https://doi.org/10.3390/nu10111567
 19. Kim H Kim B, Mun E, Jeong S, Cha Y. The antioxidant activity of steamed ginger and its protective effects on obesity induced by high-fat diet in C57BL/6J mice. *Nutrition Research and Practice*. 2018;12(6):5013-511.

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