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Anti-hyperlipidemic Effect of Date Palm on Hyperlipidemia in Rats

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ABSTRACT

Hyperlipidaemia also called high cholesterol level is an important cause of adverse health outcomes including cardiovascular complications, obesity, metabolic disorders, infertility. Date fruit is rich in phytochemicals such as carotenoids, polyphenols (e.g., phenolic acids, isoflavons, lignans, and flavonoids), tannins, and sterols. Thus with protective effect potential on hyperlipidemia. the study is design to investigate whether date palm have protective effect on margarine induced hyperlipidemia in rats. Thirty adult wistar rats weighing between 175-220g were used for this experiment. animals were divided into six groups of five rats each. group a served as control, group b was administered with margarine only. Groups C, D and E administered with margarine plus different doses of date palm extract (300, 6000 and 1200mg/kg body weight) whilst group F was administered with margarine and standard drug (atorvastatin) for eight weeks. At the end of the experiment, the wistar rats were weighed then anesthetized using chloroform. Blood tissue was collected from abdominal aorta for lipid profile analysis and differential blood count. There was significant reduction in the level of cholesterol and low density lipoprotein in groups treated with different doses of date palm extracts plus margarine compared with hyperlipidermic rats (group B). This may be due to date palm anti- hypolipidermic properties. There was an observable change in haematological parameters in some of the groups, the histopathological analysis revealed a normal myocardial, interstitial spaces and coronary vessels in all the treated groups compared to hyperlipidermic rats (group B). Date palm shows a protective effect in hyperlipidermic rats induced with margarine, thus, date palm possesses a significant anti-hyperlipidermic effect.

INTRODUCTION

It is well known that increased intakes of foods with saturated fat and cholesterol could results in high cholesterol, and subsequently raise blood cholesterol level. Such foods include egg yolks, red meat, cheese, pastries, fried and processed foods. High cholesterol level is also called hyperlipidemia.[1] Hyperlipidaemia is established to be an important cause of adverse health outcomes including cardiovascular complications, obesity, metabolic disorders, infertility.[2] Several plants can safely and effectively be used to prevent and treat some diseases. Natural plants are good remedies because they are less toxic, inexpensive and easy to access especially in the developing country like Nigeria. Date palm fruits is a plant known to possess high nutritional component like carbohydrate, protein, dietary fibers, minerals and vitamin B complex and therapeutic effect. Date palm has antioxidant, antibacterial, antifungal, and anti-proliferative properties.[3] Datapalm fruits also consist of vitamin C.[4]

Date palm (*Phoenix dactylifera*) is a flowering plant belonging to the palm family, they are berry containing a single seed enclosed by fibrous parchment. It is mostly cultivated for the consumption of its fruit. Dates palm are a major source of food and income for local populations in North and West Africa. In Nigeria, date palm is known as; esoo in Yoruba, dabino in Hausa, mkpuru ubochi in Igbo language. Flowers of date palm tree are small and yellow colored attached directly to spikelet's which develop as fruits called date palm fruits.[5]

Date fruit is used as traditional medicine in some cultures for the treatment of illnesses such as bronchitis, intestinal disorders, fever, and wound healing because of their wide variety of essential nutrient.[6] Recent studies have provided more evidence for the use of date fruits for medicinal purposes. Date fruit is rich in phytochemicals such as carotenoids, polyphenols (e.g., phenolic acids, isoflavons, lignans, and flavonoids), tannins, and sterols.[7] Antioxidants act as free radicals' scavenger associated with different diseases such as heart diseases.[8] Oxidative stress occur when the production of reactive oxygen species (ROS) is more than the capacity of the body to detoxify the reactive intermediates. The antioxidants render the free radicals to be harmless to the cells.[9] The antioxidants in the body that occur naturally which are superoxide dismutase (SOD), catalase, glutathione peroxidase (GSHPx) may not be enough to neutralize all of the free radicals generated in the body.[10]

Standard antioxidants, such as vitamin C and vitamin E

are found to have the same antioxidant activity with phenolic compounds. [11] Research has shown that date fruits have the highest concentration of total polyphenols among other dried fruits. [12] Studies has also shown that date fruits possess the second highest antioxidant activity after Hawthorn. [13]

Hyperlipidemia is an established risk factor for cardio vascular disorders (CVD), particularly atherosclerosis. CVD is one of the main reasons for premature death and is likely to be the major cause of death worldwide. [14] Dietary intake may play a vital role in the management of hyperlipidemia. Date fruits is rich in phytochemicals like phenolic acids, carotenoids, procyanidins, sterols, flavonoids and anthocyanins which is capable of lowering the level of low density lipoprotein (LDL) and glucose by decreasing its absorption through gastrointestinal tract. [15] The study was carried out to investigate the effect of date palm extract on margarine induced hyperlipidemia in rats. The study was carried out to investigate whether date palm extract has protective effect on hyperlipidemia using margarine induced hyperlipidemia rats.

Plant Materials: Raw and fresh date palm were obtained in Benin City, Edo state Nigeria. The plant was identified and authenticated by a taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city, Nigeria. The seeds were dehusked by removing the outer coat and were chopped into bits to increase surface area in order for the sample to dry quickly, then the chopped seeds were air dried and taken to the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin city, Nigeria for the aqueous extraction of date palm. After which the extract was kept in the refrigerator for use in the research.

Experimental Animals: Thirty adult Wistar rats weighing between 175-220g were used for this experiment. The rats were bred in the Animal house of the Department of Anatomy, School of Basic Medical Science University of Benin, Benin City, Nigeria. They were kept in cages in the departmental animal house where the research was carried out. The animals were acclimatized fed with growers' marsh and water. The animals were exposed to natural lighting condition and were handled according to the standard protocols for the use of laboratory animals; the weight of each animal in each group was measured weekly, so as to determine any variations in weight.

Ethical Consideration: Experimental protocol and procedures approved by the Animal ethical committee of University of Benin, Benin City, Edo State, Nigeria was used in this study and also conform to guideline in the principle of laboratory Animal care (NH 1985).

Experimental Design: Animals were divided into six groups of five rats each, as presented in Table 1. Group A which is the control group was given 1ml of distilled water, group B received margarine only while group C, D and E received margarine plus different doses of aqueous extract of the date palm respectively (300, 600, 1200 mg/kg body). Group F was administered with margarine and standard drug. This was done orogastrically using orogastric tube for six weeks.
The weight of each rats were checked and recorded before the administration of date palm extract, also repeated at the end of experiment before sacrificing at the end of six weeks of treatment. All the groups were given growers marsh and water ad libitum throughout the experiment.

Table 1: The Grouping, Treatment and the Purpose of each group.

Groups	Treatment	Extract	Purpose
Group A	1ml of distilled water	No extract	Normal control
Group B	Margarine only	No extract	Negative control
Group C	Low dose of extract plus margarine	300mg/kg body weight of extract	Protective effect
Group D	Moderate dose of extract plus margarine	600mg/kg body weight of extract	Protective effect
Group E	High dose of extract plus margarine	1200mg/kg body weight of extract	Protective effect
Group F	Standard drug plus margarine.	No extract	Therapeutic effect

Method of Sacrifice and Samples collection: At the end of the experiment, rats were weighed then anesthetized using chloroform. Blood tissue was collected from abdominal aorta and the heart by aortic and cardiac puncture respectively and was immediately transferred into plain sample bottles for lipid profile analysis and lithium heparin sample bottles for differential blood count. The heart was excised, weighed and placed in universal bottles containing 10% formalin for histopathological analysis.

Lipid Profile Analysis: The blood was collected into plain sample bottles and allowed to clot and then the clotted blood was then centrifuged and analyzed for lipid profile. Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), LDL were measured

by standard procedures using commercial analytical kit produced by Randox Laboratory Limited, UK.
This was carried out by using standard procedures and UV spectrophotometer to read the absorbance of the blank, standard and the samples, [16] while the low density lipoprotein was calculated using the formula: LDL = TC- (TG/5 + HDL).

Statistical Analysis: The results obtained from the lipid profile were analyzed. Data were represented as mean+ or –SEM (n=4). The data was analyzed using the paired sample students' T-test of the SPSS package version 17.

RESULTS

Body and Heart Weight: Results of body weight, and the mean value of heart weight following administration of date palm extract is presented in Table 2. There were no significant changes in final weights compared with initial weights in all the groups ($P > 0.05$) but there was significant increase in weight of the heart in group B, group C, group D, group E and group F compared with group A ($P < 0.05$).

Lipid Profile: In Table 3, there was significant decrease in cholesterol level in group D and E compared with group A ($P < 0.05$), but there were no significant changes in group B, C and group F compared with group A respectively ($P > 0.05$). In triglyceride level there was a significant decrease in group E compared with group A, but there were no significant changes in group B, C, D and group F compared with group A ($P > 0.05$). There were significant decreases in Very low density lipoprotein (VLDL) level in group B and group D compared with group A, but there were no significant changes in group C, E and group F compared with group A respectively ($P > 0.05$).

Blood Count: Table 4 shows the mean value of haematological indices in rats treated with different doses of date palm extracts. In white blood cell, there was a significant increase in group E compared with group A ($P < 0.05$), however, there were no significant changes in group B, C, D, and group F compared with group A ($P > 0.05$) in Table 4. In lymphocytes count and MID, there were no significant changes in all the treated groups compared with group A ($P > 0.05$). There was a significant decrease in percentage granulocytes of group E compared with group A ($P < 0.05$), however, there were no significant changes in group B, C, D, and group F compared with group A. In red blood cell and haematocrit, there were significant decrease in group B and C compared with group A ($P < 0.05$), but, there were no significant changes in group D, E and group F compared with group A. There were no significant changes in MCV in all the treated groups when compared with group A. In addition, the results of MCH and MCHC, there was a significant increase in group E compared with group A ($P < 0.05$), however, there were no significant changes in group B, C, D, and group F compared with group A ($P > 0.05$).

There was a significant increase in platelets count in group D compared with group A ($P < 0.05$), but, there were no significant changes in group B, C, E, and group F compared with group A ($P > 0.05$). There were no significant changes in PCT, MID and MCV in all the treated groups compared with group A respectively ($P > 0.05$).

Histomorphological Studies of the Heart and Blood Vessels: Figures 1-5 show the histomorphological studies of the heart and blood vessels**.** Group A, revealed a normal myocardial, interstitial spaces and coronary vessels (Fig. 1), while group B, revealed that the coronary vessels that supply the heart underwent vascular stenosis and perivascular infiltrates (Fig. 2). In group C (Fig. 3), the coronary vessels were normal with a fairly normal myocardial. In group D and E (Figs. 4 and 5), the coronary vessels and myocardial were normal.

Table 2: Body Weight, Mean Value of Heart Weight

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Body initial weight (g)	171.0 ± 6.00	174.0 ± 7.48	170.0 ± 7.42	180.0 ± 8.80	173.0 ± 4.64	185.0 ± 4.74
Body final weight (g)	162.6 ± 14.21	145.8 ± 19.63	165.4 ± 10.31	149.2 ± 15.44	156.0 ± 10.98	167.4 ± 6.30
*Heart weight (g)	0.46 ± 0.11	1.05 ± 0.07	1.02 ± 0.07	1.04 ± 0.06	1.12 ± 0.10	1.13 ± 0.06

$P < 0.05$ indicates significant difference. *Determined after administration of date palm extract.

Table 3: Plasma Lipid Profile

Parameters	Group A	Group B	Group C	Group D	Group E	Group
CHOL (mg/dl)	164.8 ± 22.25	144.7 ± 10.13	127.6 ± 15.10	93.52 ± 10.61*	103.4 ± 11.51*	96.57 ± 21.37
TG (mg/dl)	115.2 ± 13.21	67.31 ± 5.28*	75.71 ± 14.23	63.14 ± 7.61*	81.65 ± 8.92	89.68 ± 10.07
HDL (mg/dl)	58.82 ± 3.88	63.92 ± 4.15	67.09 ± 6.23	53.48 ± 10.90	37.90 ± 6.48*	42.92 ± 9.05
LDL (mg/dl)	82.92 ± 23.05	67.30 ± 8.80	45.36 ± 9.27	27.42 ± 4.27	50.43 ± 7.30	35.72 ± 11.53
VLDL (mg/l)	23.04 ± 2.64	13.46 ± 1.06*	15.14 ± 2.85	12.63 ± 1.52*	16.33 ± 1.78	17.94 ± 2.01

*P < 0.05 indicates significant difference in treated groups compared with the control.
CHOL: Cholesterol, TG: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein.
Group A: Normal rats given distilled water

Table 4: Mean Value of Haematological Indices in Rats Treated with Different Doses of Date Palm Extracts

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
WBC count ($x10^3/\mu l$)	3.360 ± 0.39	3.560 ± 0.27	3.633 ± 0.45	4.875 ± 0.79	5.540 ± 0.71*	3.667 ± 1.36
Lymphocytes	67.26 ± 15.80	86.62 ± 1.56	86.43 ± 2.89	89.25 ± 2.03	90.36 ± 0.76	89.90 ± 2.36
MID	10.96 ± 3.06	7.940 ± 0.82	7.933 ± 1.35	6.525 ± 1.00	6.900 ± 0.68	6.533 ± 1.89
GRAN	8.180 ± 2.11	5.440 ± 1.29	5.633 ± 1.55	4.225 ± 1.62	2.740 ± 0.17*	3.567 ± 1.29
RBC count ($x10^6/\mu l$)	7.488 ± 0.09	6.456 ± 0.29*	6.433 ± 0.26*	6.703 ± 0.48	7.728 ± 0.24	6.910 ± 0.39
Haemoglobin (g/dl	14.76 ± 0.20	12.26 ± 0.82*	12.60 ± 0.52*	13.60 ± 0.82	15.80 ± 0.54	13.57 ± 0.89
Haematocrit (%)	45.56 ± 0.91	37.98 ± 1.89*	38.13 ± 1.09*	41.55 ± 2.19	46.36 ± 1.49	40.80 ± 2.60
MCV (fl)	60.90 ± 0.57	58.92 ± 1.27	59.47 ± 1.30	62.40 ± 1.78	60.06 ± 0.25	59.10 ± 0.75
MCH (pg)	19.68 ± 0.16	18.86 ± 0.56	19.53 ± 0.27	20.33 ± 0.50	20.38 ± 0.11*	19.57 ± 0.27
MCHC (g/dl)	32.36 ± 0.44	32.14 ± 0.84	32.97 ± 0.48	32.65 ± 0.25	34.02 ± 0.27*	33.20 ± 0.70
Platelet count	480.8 ± 85.87	617.0 ± 79.52	386.0 ± 163.7	770.3 ± 36.00*	710.8 ± 77.61	682.3 ± 88.06
PCT (%)	7.760 ± 0.15	7.920 ± 0.20	6.067 ± 1.94	8.250 ± 0.25	7.700 ± 0.15	7.267 ± 0.12

*P < 0.05 indicates significant difference in treated groups compared with the control

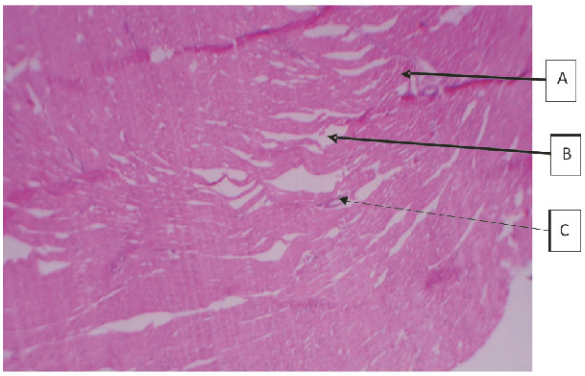

Figure 1: Control; A represents bundles of cardiomyocytes, B represents interstitial space, and C represents coronary artery (H&E x 40)

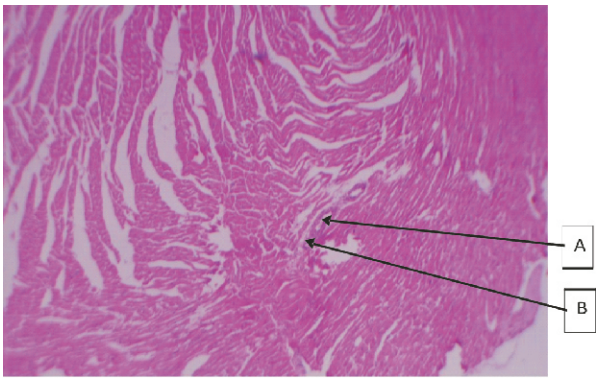

Figure 2: Margarine only: A represents vascular stenosis, and B represents penvascular infiltrates of inflammation (H&E x 40).

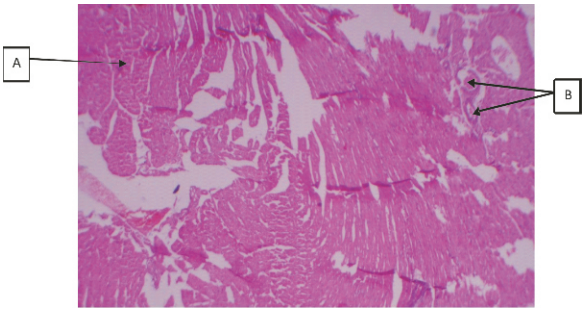

Figure 3: Margarine plus low dose extract: A represents normal myocardiac, and B represents vascular architecture (H&E x 40).

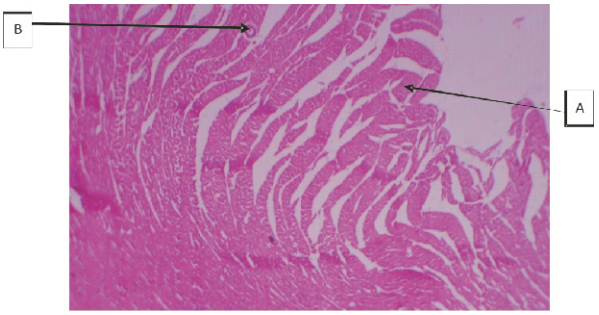

Figure 4: Margarine plus moderate dose extract: A represents normal myocardiac, and B represents vascular architecture (H&E x 40).

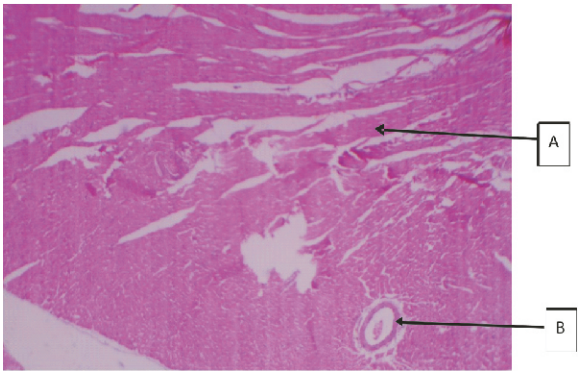

Figure 5: Margarine plus high dose extract: A represents normal myocardiac, and B represents vascular architecture (H&E x 40).

DISCUSSION

Daily intake of diet rich in fruits and vegetables has been shown to protect against lipid peroxidation. [17] Studies has also shown that date fruits have the highest concentration of total polyphenols among other dried fruits considered in [18]. The antioxidant activity of phenolic compounds in date fruit is found to be comparable to the standard antioxidants, such as vitamin C, vitamin E, and β-carotene. [19]

Increase in cholesterol level is a major cause of coronary heart disease and also results to the process by which the blood vessels that supply the heart and other parts of the body become blocked. [20] The decrease in the

level of cholesterol by date palm extracts with different doses showed a protective effect against hyperlipidermia

Research in the current study using seeds extracts of date palm (*Phoenix dactylifera*) on margarine induced hyperlipidemia rats, show that there was significant reduction in the level of cholesterol and low density lipoprotein. Flavonoids and Vitamin C are richly present in date fruit *(Phoenix dactylifera*). [4] These antioxidant constituents of date palm *such as* flavonoids and vitamin C may directly react with ROS to destroy them by donating electrons to eliminate the unpaired form of ROS. Hyperlipidemia is the main risk factor for atherosclerosis that leads to death. Scientists are in great search of new drug with little or no side effect to counter hyperlipidemia. Date palm (which is from plant) may be a good alternative traditional drug for the treatment of hyperlipidemia

There are many mechanisms by which herbs can reduce hyperlipidemia, from the current study there was inhibition of lipid production as seen in the reduction in the level of cholesterol and LDL. Most studies transmit the pharmacological potentials of date palm to its antioxidant activity. Therefore, decreases in the level of cholesterol, triacylglycerol and VLDL in some of the groups treated with date palm extract may be due to flavonoid and vitamin C content in date palm. Thus, the combined effects of folate, flavonoids, vitamin C and polyphenolic antioxidants in the date palm may be responsible for its protective effect. [21]

Experimental investigation on cardioprotective effect of lyophilized aqueous date fruit extract *in-vivo and ex-vivo*, discovered that it enhances the cardiomyoblast cell proliferation by up to 40% which prevented the consumption of endogenous antioxidants and inhibited lipid peroxidation, [22] which is consistent with the current study. Also, histomorphological studies of the heart and blood vessels in the current study showed that administration of date palm extract reduced vascular stenosis and perivascular infiltrates of inflammation.

Conclusion

This paper has focused on the potential protective effect of date palm extract on hyperlipidemia in margarine induced hyperlipidermic rats. The experimental procedure carried showed that high cholesterol diet like (margarine), had severe damages to the coronary vessels and consequent myocardial injury, although the damages that occurred in the blood vessels might not be severe enough to cause myocardial infarction. However, date palm extract shows a protective effect by decreasing level of cholesterol and low-density lipoprotein.

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