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Research Article
Extra Virgin Olive Oil and Palm Oil Diets Reduce Blood Pressure via K_{atp}/B_{kca} Ion Channels in Rats

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Abstract

Background and Objective: Extra virgin olive oil is consumed in most areas of the Mediterranean and the tropics and has been reported to be involved in the lowering of arterial blood pressure. The aim of this study was to determine the possible mechanism of arterial blood lowering effect of extra virgin olive oil and palm oil diets. **Materials and Methods:** Male Wistar rats (10 rats per group) were fed on normal rat chow (control), Fresh Palm Oil (FPO), Extra Virgin Olive Oil EVOO) and Thermoxidized Palm Oil (TPO) groups. The oil-fed groups received 15% (weight/weight) of oil mixed with rat chow for 18 weeks. Blood pressure was measured by direct cannulation and blood samples, heart and kidney were collected for biochemical analysis. **Results:** The basal Systolic Blood Pressure (SBP) and after phenylephrine (10^{-6} mmol L^{-1}) infusion was significantly ($p<0.01$) elevated in EVOO, FPO and TPO compared with control. Levcromakalim (10^{-6} mmol L^{-1}) (a K_{ATP}-channel opener) and NS1619 (B_{kca} channel opener) infusion significantly ($p<0.01$) reduced mean arterial pressure (MAP) in FPO and EVOO compared with control and TPO. Heart and kidney SOD and catalase activities were significantly ($p<0.05$) increased in EVOO and FPO groups when compared with other groups but decreased in TPO. **Conclusion:** Long term intake of thermally oxidized palm oil may suppress potassium ion channel function thereby increasing blood pressure. Blood pressure reduction property in FPO and EVOO is via K_{ATP} and B_{kca} ion channels and the greater effect of EVOO is probably due to its high content of oleic acid and polyphenols.

Key words: Antioxidant, blood pressure, levcromakalim, NS1619, olive oil, palm oil, phenylephrine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Unrefined extra virgin olive oil is consumed predominantly by people around the Mediterranean areas[1]. It is obtained from *Olea europaea* and has high content of polyphenol, oleic acid (n-9) and it is less susceptible to oxidation[2]. Olive oil is a major source of dietary fat[3] and it has some therapeutic properties important in prevention of cardiovascular disease[4]. Palm oil is consumed worldwide particularly in Malaysia[5] and most tropical countries like Nigeria. Human and animal studies have demonstrated that the consumption of monounsaturated and polyunsaturated fatty acids such as olive oil, coconut oil and palm oil can lead to a decrease in blood pressure[6,7] and protect against the development of hypertension. The use of olive oil for culinary purposes has increased due to awareness that the oil is rich in micronutrients and monounsaturated fatty acids such as oleic acid[8], polyphenols, tyrosol, hydroxytyrosol, oleuropein, listrosides, flavonoid and squalene[2,9]. All these components have beneficial role in blood pressure reduction, scavenging and protecting heart tissues, oxidative stress and serum lipid reduction[10].

Palm oil on the other hand is obtained from the pulp of the fruit of the oil palm *Elaeis guineensis*. It also contains a high proportion of saturated palmitic acid, monounsaturated oleic acid as well as linoleic acids, α and β-carotenes vitamin C, tocopherols and tocotrienols[11,12]. These constituents are known anti-oxidants and protect against lipid peroxidation and oxidative stress[13], cell membrane protein oxidation and lower blood pressure[14,15].

It has been a common practice (mostly by food vendors) to reuse oils used for frying thus subjecting it to thermal oxidation, hydrolysis and polymerization due to exposure to high temperature. The repeated heating of cooking oil makes the oil more prone to lipid peroxidation[16] and when these foods are taken, the toxic products, such as hydroperoxides and aldehydes are absorbed into the body system and the potential effect with this is usually harmful. This could be increased blood vessel contraction and arterial blood pressure as well as DNA damage[5,17,18].

Apart from the various established mechanisms for the regulation of arterial blood pressure, there is paucity of information on the involvement of dietary oils and ion channels in the etiology of cardiovascular diseases. Research has shown that the opening and closing of ion channels tends to modulate cell homeostasis and protect against cell damage. Amongst the channels widely studied is the potassium ion channels. These channels are involved in vascular relaxation and are known to be altered in disease state such as hypertension and diabetes mellitus[19]. A recent study reported that altered K_{ATP} and B_{kca} ion channel function is one of the mechanisms involved in increased blood pressure by thermally oxidized palm oil[20]. Therefore, in this study, it aimed at investigating the involvement of B_{kca} and K_{ATP} potassium ion channel in the reduction of arterial blood pressure in Wistar rats fed with extra virgin olive oil and palm oil diet.

MATERIALS AND METHODS

Drugs: Glibenclamide (10^{-5} mmol L^{-1}), Levcromakalim (10^{-6} mmol L^{-1}), 0.1% Dimethyl sulfoxide (DMSO), Phenylephrine (10^{-7}), NS1619 (1,3-Dihydro–1-[2-hydroxy-5-(trifluoromethyl) phenyl]-5-(Trifluoromethyl) 2H-benzimidazol-2-one) (10 mmol L^{-1}) and urethane were obtained from Sigma Chemical Co., St. Louis, MO, USA. Levcromakalim and NS1619 were dissolved in DMSO and diluted with normal saline before infusion.

Experimental animals and design: Sixty male Wistar rats weighing between 180-250 g were obtained from the animal house of Physiology Department, Cross River University of Technology, Calabar, Nigeria. The animals were kept in plastic cages and at room temperature of 28±2°C with 12 h light/dark cycle. Sixty male Wistar rats were randomly grouped into four of 15 rats each. Group 1 (Control group) received normal rat feed. Group 2 (FPO), Group 3 (EVOO) and Group 4 (TPO) received 15% (weight/weight) of fresh palm oil, extra virgin olive oil and five times heated palm oil, respectively for 16 weeks. All groups had free access to water and rat chow *ad libitum*. Ethical approval for the study was obtained from the Faculty of Basic Medical Sciences Animal Research Ethical Committee of the University of Calabar, Calabar (approval number FBMS/CMS/UNICAL/15/020).

Preparation of oil diets: Unrefined (fresh) palm oil and extra virgin olive oil (EVOO) used for this study were purchased from Okuku market, in Yala Local Government and the Rafael Salgado, Cira, Madrid, Spain, respectively. Palm oil used was fresh or heated five times with modification as previously described[21]. The diet was formulated by mixing 15% (w/w) of oil with the rat chow, respectively.

Measurement of blood pressure and heart rate: Each rat was anaesthetized with 8 mg kg^{-1} b.wt., of 15% urethane intraperitoneally. The tracheal ring was cut opened and cannulated to improve ventilation while the jugular vein was

cannulated for infusion of drugs. The carotid artery was cannulated and connected to a pressure transducer (Statham P23 XL) which was in turn connected to a Supertek Physiograph for arterial blood pressure recording. Mean arterial pressure (MAP) was calculated as DBP+1/3 (pulse pressure).

To characterize the involvement of potassium ion channel after the measurement of basal arterial pressure in each animal, levcromakalim (10^{-6} mmol L^{-1}) or NS1619 (10 mmol L^{-1}) was infused after phenylephrine (10^{-7} mmol L^{-1}) injection. The changes in systolic, diastolic pressures and heart rate were measured and compared with control group.

Collection of blood samples: In another set of experiment, the animals were anaesthetized and blood samples were collected by cardiac puncture into sterile plain bottles. Each blood sample was allowed to clot and then centrifuged at 2000 g for 10 min to obtain serum. The serum was stored at -20°C till further use.

Preparation of tissue homogenates and biochemical assay: Heart and kidney were removed immediately, trimmed of fats and connective tissues and rinsed in ice-cold 5% KCl solution. The tissues were homogenized in 0.1 M potassium phosphate buffer (pH 6.5) and then centrifuged in cold ice at 10,000 g for 10 min in order to obtain a post mitochondria fraction. Superoxide dismutase activity was determined by the method of Misra and Fridovish[22] while catalase enzyme activity was determined using the method of Sinha[23]. Malonylaldehyde (MDA), a stable product of lipid peroxidation was estimated by the method of Ohkawa *et al.*[24].

Statistical analysis: All results were presented as mean±standard error of mean (SEM). One way analysis of variance (ANOVA) was done using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, Ca, USA). Comparison test was done using Bonferroni multiple test and probability level of $p<0.05$ was considered as significant.

RESULTS

Basal blood pressure and heart rate in the presence of phenylephrine: At the end of 18 weeks, basal mean arterial pressure in thermally oxidized palm oil (TPO) group was significantly ($p<0.05$) elevated compared to the control, EVOO and fresh palm oil (FPO)-fed groups (Table 1). There was no significant difference in MAP of rats in the control, EVOO and FPO groups. Phenylephrine infusion significantly ($p<0.05$) increased SBP and DBP in EVOO, FPO and TPO. The heart rate was significantly ($p<0.05$) elevated in TPO group compared to other experimental groups.

Effect of levcromakalim and NS1619 on blood pressure and heart rate in the presence of phenylephrine: The MAP reduction of by levcromakalim in EVOO-fed groups was 24% which was significantly ($p<0.01$) high compared with FPO-fed group (Fig. 1). The MAP reduction in the control, FPO, EVOO and TPO were 11, 12, 20 and 6.4%, respectively. The MAP reduction by NS1619 was significantly ($p<0.05$) higher in EVOO than FPO and control groups. The heart rates following levcromakalim and NS1619 infusion in EVOO and FPO-fed groups were significantly ($p<0.05$) reduced when compared with phenylephrine infusion and basal values.

Tissue peroxidation levels, superoxide dismutase and catalase activity: Tissue peroxidation level, superoxide dismutase and catalase activity in the heart and kidney are shown in Table 2. There was a significant ($p<0.01$) increase in peroxidation in TPO in the heart and kidney while EVOO and FPO supplementation led to a significant ($p<0.01$) reduction when compared to control. Superoxide dismutase activity in

Table 1: Basal arterial blood pressure and heart rates in rats treated with extra virgin olive oil and palm oil in the presence of phenylephrine

Parameters	Control	FPO	EVOO	TPO
Basal blood pressure (mmHg)				
Systolic BP (mmHg)	90±2	86±2	80±2^{+}	150±3**
Diastolic BP (mmHg)	77±2	70±2	70±1	120±4*
MAP (mmHg)	81.3±1	75±0.7^{+}	73.3±1^{+}	130±2**^{a}
Heart rate (beats/min)	360±5	350±5	355±5	460±6*
Phenylephrine (PHE) infusion				
Systolic BP (mmHg)	100±2	106±3	110±3	160±4*
Diastolic BP (mmHg)	81±2	83±3	80±3	135±4*
MAP (mmHg)	87±2	90±1	90±1	143±2**
Heart rate (beats/min)	340±2	330±4	342±5	440±6**
Heart rate in the presence of levcromakalim (beats/min)	336±3	320±6	328±4	420±6
Heart rate in the presence of NS1619 (beats/min)	330±3	331±4	322±6	431±6

Values are expressed as mean±standard error of the mean (SEM), n = 6 in each group, MAP: Mean arterial pressure, BP: Blood pressure, FPO: Fresh palm oil, TPO: Thermally oxidized palm oil, **p <0.01 vs. control, +: p<0.05 vs. control, a: p<0.01 vs. EVOO and FPO

Tables 2: Tissue lipid peroxidation and anti-oxidant activity in control and experimental groups fed on dietary oils

Organ/anti-oxidant parameter	Control	FPO	EVOO	TPO
Peroxidation (mol g^{-1} tissue)				
Kidney	1.56±0. 02	1.43±0.1*	1.46±0.2*[a]	2.84±0.1**
Heart	1.24x10^{-5}±0.08	1.23x10^{-5}±0.04	1.22 x10^{-5}±0.2[a*]	2.60x10^{-5}±0.3 *
SOD (mg/mol/protein)				
Kidney	0.21±0.03	1.25±0.02*	1.38±0.03*[a]	0.14±0.03[b]
Heart	0.24±0.03	0.48±0.05*	0.75±0.2*[a]	0.25±0.05[b]
Catalase (mg/mol/protein)				
Kidney	0.79±0.02	0.60±0.02*	0.88±0.01*[a]	0.30±0.01[b]
Heart	0.39±0.02	0.41±0.02	0.49±0.02*	0.20±0.01[b]

Values are expressed in mean±SEM, n = 6, *p<0.05 vs. control, **p<0.01 vs. control, a: p<0.01 vs. FPO, b: p<0.05 vs. FPO and EVOO

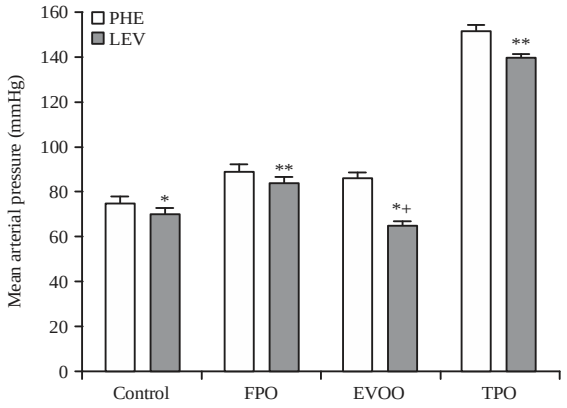

Fig. 1: Effect of levcromakalim on phenylephrine-induced mean arterial pressure in rats fed on various dietary oil
FPO: Fresh palm oil-fed group, EVOO: Extra virgin olive oil-fed group, TPO: Thermally oxidized oil-fed group, n = 6, *p<0.05 compared with PHE, **p<0.01 compared with PHE, +: p<0.05 compared with FPO

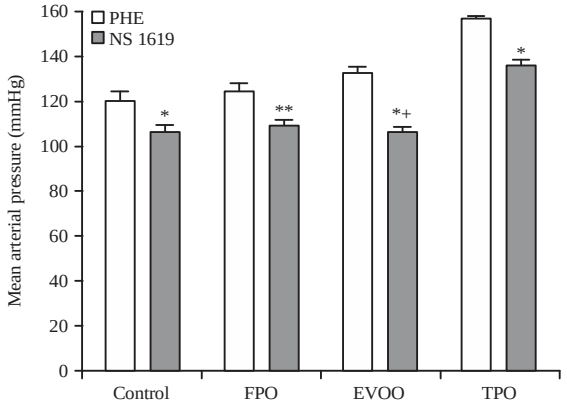

Fig. 2: Effect of NS1619 on phenylephrine-induced mean arterial pressure in rats fed on various dietary oil
FPO: Fresh palm oil-fed group, EVOO: Extra virgin olive oil-fed group, TPO: Thermally oxidized oil-fed group, n = 6, *p<0.05 compared with PHE, **p<0.01 compared with PHE, +: p<0.05 compared with FPO

the kidney and heart tissues in EVOO and FPO groups were significantly (p<0.05) increased when compared to control. Catalase activity in the kidney of EVOO and FPO-fed groups were significantly (p<0.05) raised while it was significantly (p<0.01) decreased in TPO fed group compared with control. In all cases, there was a significant improvement in biochemical parameters in EVOO-diet group when compared with FPO-diet group.

DISCUSSION

This study was aimed at finding the possible mechanism of blood pressure reduction *in vivo* by EVOO and palm oil. To achieve this objective, both potassium ion channel blockers and openers were used. The results obtained in this study showed a significant beneficial reduction in basal MAP in rats fed on EVOO and FPO diets and a significant increase in TPO when compared with control. This is in agreement with previous studies on the effect of dietary supplementation of fresh palm oil diet on blood pressure[15,25,26].

The administration of phenylephrine significantly increased basal SBP and DBP in all the experimental groups. Phenylephrine acts majorly via the α_1-adrenoreceptors leading to vasoconstriction and increase in blood pressure[27]. Interestingly, the EVOO and FPO groups showed significant decrease in both systolic and diastolic blood pressures though it was higher in TPO in this *in vivo* study. This observation collaborates with a previous *in vitro* study that reported a reduced contraction response of FPO to phenylephrine[25]. The reason for the observed increase in the fresh oil and EVOO *in vivo* can be attributed to the high content of oleic acid which have been reported to cause a reduction in blood pressure[28] by enhancing the vasodilatory $\alpha_{2A/D}$-adrenoreceptor/G protein/cyclase-cAMP/PKA pathway[29]. When guanylate cyclase is stimulated, it results in an elevated cyclic guanosine monophosphate (cGMP) formation leading to vascular smooth muscle relaxation[30] and a reduction in blood pressure.

The blood pressure reducing ability of fresh palm oil and extra virgin olive oil is attributed to the anti-oxidant found in

EVOO and tocopherols, tocotrienols, flavonoids, vitamin C and vitamin E that are present in palm oil which enhance nitric oxide concentrations leading to vasodilation[2,31]. All of these antioxidants are biologically active compounds involved in scavenging free radical and reduction of blood pressure[32]. The EVOO diet caused a greater reduction of blood pressure than FPO-diet in this study. This may probably be due to increased content of phenols and high content of oleic acid. A study has shown that EVOO contains 70-80% oleic acid[29] while the percentage composition of oleic acid in palm oil was reported[33] to be 40-50%.

Generally, heating oils at high temperatures causes lipid peroxidation and generation of free radicals anions[25,31]. These free radicals react with NO to form peroxynitrite[34] causing a reduction in plasma nitrite levels and loss of NO-dependent relaxation. The reduction in NO bioavailability and reduced radical-scavenging properties could have possibly contributed to an increase in BP in TPO-fed rats compared to the control and other dietary oil-fed groups[31].

It is noteworthy that administration of levcromakalim, a K_{ATP} ion channel opener and NS1619 (B_{kca} channel opener) led to a significant decrease in MAP in EVOO and FPO-fed groups. These results suggested that potassium ion channels play a role in the regulation of blood pressure. It therefore suggests that K_{ATP} and B_{kca} channels opening may be a possible mechanism of blood pressure reduction by EVOO and FPO. NS1619 and levcromakalim administration in this study could not reduce MAP in the TPO group thus suggesting suppression of K_{ATP} and B_{kca} channel activity due to thermal oxidation. This outcome pointed at potassium ion channel insensitivity to producing a decrease in blood pressure. Usually, thermal oxidation products alter the sensitivity of the phospholipid membrane and its electrical property as well as impairment of endothelium-dependent vasorelaxation[35,36]. Though previous studies reported that palm oil[37,38] and extra virgin olive oil[39] reduce blood pressure through enhanced vasodilation, no study has investigated the involvement of potassium ion channel with respect to these two dietary oil. Our present study shows that EVOO and palm oil may also lower blood pressure via activation of the K_{ATP} and B_{kca} channels.

Furthermore, in this study, we found a significant reduction in lipid peroxidation in the heart and kidney from the extra virgin olive oil and fresh palm oil groups and a concomitant increase in proportion of the SOD and catalase enzyme activity in these tissues. This indicated a strong antioxidant activity and a protective function against cellular and tissue damage[40]. This enhanced SOD activity may be due to the presence of β-Sitosterol in EVOO which is reported to increase SOD activity and decreases superoxide level[41]. A limitation is that this study did not estimate the expression of these ion channels. Further study is needed on the K_{ATP} and B_{kca} ion channel expression in the blood vessels and the heart to further characterize the mechanism of action of these dietary oil.

CONCLUSION

The present results showed that long term intake of thermally oxidized palm oil may suppresses potassium ion channel function thereby inhibiting blood pressure response to potassium ion channel openers. It concluded that blood pressure reduction caused by EVOO and FPO is via the activation of K_{ATP} and B_{kca} channels and probably due to high content of antioxidants.

SIGNIFICANCE STATEMENT

This study discovered the extra virgin olive oil and fresh palm oil causes a reduction of blood pressure through the activation of K_{ATP} and B_{kca} potassium ion channel that can be beneficial for the management of hypertension. This study will help the researchers to uncover the critical areas of ion channel that many researchers were not able to explore. Thus a new theory on potassium ion channel involvement in management of blood pressure by dietary method may be arrived at.

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