Journal of Medicinal Plants Research Vol. 6(18), pp. 3501-3505, 16 May, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.202
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

The hypolipidemic and antioxidant actions of aqueous extracts of *Ocimum basilicum* and *Ocimum suave* in high fat fed Rats

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Accepted 28 March, 2012

The abilities of *Ocimum suave* (OS) and *Ocimum basilicum* (OB) to prevent high fat diet (HFD) induced hyperlipidemia and oxidative stress in Wister albino rats was investigated. Groups of rats maintained on a HFD, containing 31% fat, were given daily oral doses of 800 mg/kg of extracts of *O. suave* or *O. basilicum* for 21 days. High fat feeding caused significant ($p < 0.05$) increases in serum levels of total, HDL and LDL cholesterol, but significantly ($p < 0.05$) reduced the serum triacylglycerols in the HFD control rats, as compared with the rats fed normal feed (7% fat). Administration of the aqueous extract of *O. suave* or *O. basilicum* to HFD fed rats significantly ($p < 0.05$) prevented the HFD induced increases in serum total, HDL and LDL cholesterol, while partially, though significantly, prevented the HFD induced decrease in serum triacylglycerols. The effects of the two extracts were comparable with that of the standard hypolipidemic drug (Lipitor®). The HFD induced significant ($p < 0.05$) increase in serum Albumin levels was unaffected by all treatments. The HFD control rats had significantly ($p < 0.05$) elevated levels of thiobarbituric acid reactive substances (TBARS**) in the serum, liver and kidney; as compared with normal controls. The extracts prevented these increases. Serum and kidney superoxide dismutase (SOD) activities dropped significantly when HFD only was fed to rats; the liver SOD was unaffected. Administration of the aqueous extracts of *O. suave* to HFD fed rats caused the SOD activities in the serum, liver and kidney to rise significantly above even the levels recorded in rats fed normal feed. It was concluded that the hyperlipidemia induced by HFD feeding was prevented by aqueous extracts of *O. suave* and *O. basilicum*; and that high fat diet (HFD) increases oxidative stress which was ameliorated by concomitant administration of the extracts.**

Key words: Hyperlipidemia, high fat diet, *Ocimum suave*, *Ocimum basilicum*, oxidative stress, antioxidant.

INTRODUCTION

Hyperlipemia is a condition which can be found to be associated with overweight and obesity. Overweight and obesity are the main risk factors in diseases such as hypertension, non-insulin dependent diabetes mellitus, gallbladder and other types of cancer (Ortiz-Moreno et al., 2007). There is, however evidence that, genetics, dietary factors and nutritional habits influence the risk of cardiovascular diseases (Abdulazeez, 2011). It has been established that "Western diets," known for their high fat, high cholesterol, excess energy, and low fiber contents, increase serum cholesterol and other lipid levels (Jingjing and Xiangrong, 2007), which usually predisposes the individual to the aforementioned complications and diseases. It has been reported that complications and diseases associated with hyperlipidemia cause almost 12 million deaths each year all over the world (Bakari et al., 2007).

The major risk due to hyperlipidemia is related to atherosclerosis and one of the initial events in this process is the accumulation of cells containing excess lipids within the arterial wall (Chisolm, 2001). Hyperlipidemia also plays the further role of stimulating the production of

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reactive oxygen radicals from polymorphonuclear leukocytes and monocytes (Wilson and Gelb, 2002), thus oxidative stress is associated with hyperlipidemia. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases (Terman et al., 2007). Many researchers have focused on natural antioxidants; and in the plant kingdom numerous crude extracts and pure natural compounds, including some from the *Ocimum* species, were previously reported to have antioxidant properties (Muthu et al., 2010).

Basil (*Ocimum* spp. L) is a small shrub with many branches, commonly found in many gardens around village huts in Nigeria and planted for its medicinal uses. Originating from Central Africa and South East Asia, basil is probably one of the most commonly used of all herbs in cooking and there are many recipes, local and foreign, that are enhanced by this minty aromatic plant. In Nigeria, about four varieties of basil are known to occur naturally in Southern and Northern parts of the country. These are *Ocimum suave, Ocimum gratissimum, Ocimum basilicum* and *Ocimum camium*. Local names of basil amongst various ethnic groups include: "Efirin" (Yoruba), "Neh – anwu" (Ibo), "Ntion" (Efik) and "Dan-doya ta gida" (Hausa).

Traditionally, basil has been used as a medicinal plant in the treatment of headache, diarrhea, wart, worms and kidney dysfunction (Seung-Jo-Lee et al., 2004). It is also being used in the treatment of diabetes and cardiovascular diseases (Zeggwagh et al., 2007). In addition to health benefits, basil is used widely as a condiment or spice and as a source of flavor in soup preparations (Mohammed et al., 2007). Thus, this paper investigates the hypolipidemic and antioxidant actions of the aqueous extracts of *O. basillicum* and *O. suave* in diet induced hyperlipidemic rats as a way of validating its folkloric use.

MATERIALS AND METHODS

Plant collection and extraction

The leaves of *O. basillicum* and *O. suave* were harvested from gardens around Samaru, Zaria in the month of March. Identification was done at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, a voucher number was deposited. Shade dried leaves were pulverized and 100 g of the powdered leaves was soaked in 1 L distilled water for 24 h, with intermittent shaking. The suspension was filtered first through muslin cloth and then Whatman's No 1 filter paper. The filtrate was evaporated to dryness and the extract reconstituted in distilled water when required.

Feed formulation

Commercial Grower's mash (Vital Feeds, Jos) was used as the normal feed and its proximate composition was: Carbohydrate 66.65%, lipid 7.00%, proteins 15%, crude fiber 10%, phosphorus 0.35% and calcium ion 1.00%. The high fat feed was formulated by adding Baker's fat to the aforementioned feed to obtain the following composition: Carbohydrate 49.37%, lipid 31.11%, proteins 11.11%, crude fiber 7.41%, phosphorus 0.003%, and calcium ion 0.007%.

Animal treatment

Thirty five male Wister strain albino rats (120 to 150 g) were acclimatized to the laboratory conditions for two weeks. The rats were then randomly divided into seven groups of five rats each. Three groups were maintained on normal feed and drinking water *ad libitum* for the three weeks duration of the experiment. Two of these groups were each given daily oral dose of 800 mg/kg aqueous extract of *O. basilicum* or *O. suave*. The remaining group was maintained without further treatment. Four other groups were maintained on the high fat feed and drinking water *ad libitum*; two of these were similarly treated with the extracts of *O. basilicum* or *O. suave*. One high fat fed group was given daily oral dose (167 µg/kg) of a standard hypolipdemic drug (Lipitor®) and the remaining high fat fed group was kept without further treatment.

All animals were humanely sacrificed in the fasted state on the 22nd day, post treatment, and blood, liver and kidneys collected.

Sample preparation and analysis

Serum was harvested from the blood and stored at -20℃ until required. One gram of organ was homogenized in 20 ml of 0.01 M phosphate buffer, pH 7.4; and centrifuged at 3000 × g to collect the supernatant, which was used as organ extract. The assays of total, HDL, and LDL cholesterol, total protein and albumin as well as glucose in the serum were done using commercial reagent kits (Agappe Hills' Kerala, India). Alanine transaminase (ALT) and aspartate transaminases (AST) as well as Urea were assayed in the serum using commercial reagent kits (Randox Laboratories, UK). Thiobarbituric acid reactive substances (TBARS), assayed as Malondialdehyde, was determined using the method described by Ohkawa et al. (1979). Catalase and Superoxide dismutase activities were assayed by methods described by Machly and Chance (1954) and Misra and Fridovich (1972), respectively.

Statistical analysis

Data are presented as Means±SD and analyzed using ANOVA and Ducan post hoc test and significance was determined at $p < 0.05$.

RESULTS

The feed intake data in Table 1 shows that the various treatments did not significantly affect the quantity of feed consumed by the groups. High fat diet (HFD) caused significant ($p < 0.05$) increases in serum total, HDL, and LDL cholesterol levels but significantly decreased the serum triacylglycerols levels. Separate administration of the two extracts to HFD rats significantly prevented the HFD induced increases in the above mentioned parameters; with *O. suave* being more effective than *O. basilicum*. The decrease in triacylglycerols levels observed in the untreated HFD group was significantly ($p < 0.05$) reversed by administration of either of the

Table 1. Feed intake and change in body weight of rats fed a normal or high fat feed and treated with aqueous extracts of *O. basilicum* or *O. suave*.

Parameter	Normal control[a]	Normal + *O. basilicum*[a]	Normal + *O. suave*[a]	HFD control[a]	HFD + *O. basilicum*[a]	HFD + *O. suave*[a]	HFD + Lipitor[a]
Feed intake (g/100g/day)	25.84 ± 1.48	25.56 ± 0.85	25.25 ± 1.50	24.61 ± 1.44	27.22 ± 0.40	25.85 ± 0.83	25.78 ± 1.52
Increase in body wt (%)	8.19 ± 5.70	8.81 ± 7.26	12.98 ± 6.75	9.98 ± 3.89	11.50 ± 5.51	14.10 ± 4.62	16.11 ± 6.33

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

Table 2. Effect of aqueous extracts of *O. basilicum* and *O. suave* on serum lipid profiles, total proteins, albumin, and blood glucose in rats fed normal or high fat diet.

Parameter	Normal control	Normal + *O. basilicum*	Normal + *O. suave*	HFD Control	HFD + *O. basilicum*	HFD + *O. suave*	HFD + Lipitor
Total cholesterol (mg/dl)	420.00 ± 98.99^{ac}	390.00 ± 42.43^{a}	1108.00 ± 82.58^{b}	1183.33 ± 96.00^{b}	942.00 ± 172.82^{b}	560.00 ± 117.69^{c}	296.67 ± 50.17^{d}
Triacyl-glycerols (mg/dl)	377.84 ± 80.29	44.12 ± 5.10^{c}	151.53 ± 20.41^{b}	77.94 ± 14.63^{d}	200.00 ± 12.48^{e}	135.29 ± 15.00^{b}	91.86 ± 9.80^{f}
HDL-Cholesterol (mg/dl)	75.00 ± 17.68^{a}	130.00 ± 11.18^{c}	181.75 ± 32.48^{b}	150.00 ± 17.68^{c}	256.25 ± 10.82^{d}	83.33 ± 10.21^{a}	166.67 ± 10.21^{b}
LDL-Cholesterol (mg/dl)	145.56 ± 14.28^{a}	60.82 ± 8.54^{c}	207.26 ± 14.34^{b}	222.25 ± 13.08^{b}	185.15 ± 33.46^{b}	122.41 ± 24.25^{a}	71.40 ± 14.53^{c}
Total protein (g/L)	119.00 ± 3.55^{a}	158.18 ± 13.99^{b}	125.33 ± 3.10^{a}	112.00 ± 13.44^{a}	104.50 ± 8.81^{a}	128.47 ± 11.91^{a}	160.36 ± 12.37^{b}
Albumin (g/dl)	15.51 ± 0.42^{a}	29.96 ± 5.98^{b}	33.32 ± 4.11^{b}	37.25 ± 2.83^{b}	30.28 ± 1.31^{b}	36.51 ± 3.20^{b}	32.67 ± 3.75^{b}
FBG (mM)	4.01 ± 1.35^{ab}	3.88 ± 0.46^{a}	4.65 ± 0.23^{ab}	4.48 ± 0.26^{ab}	6.95 ± 0.77^{b}	4.35 ± 0.29^{ab}	4.47 ± 0.42^{ab}

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

extracts to HFD rats; in this regard, however, *O. basilicum* appeared to be more effective than *O. suave*. The lipid – lowering effect of the standard hypolipidemic drug (Lipitor®) given to HFD fed rats was significantly higher than that exhibited by both extracts. Although HFD caused no significant ($p > 0.05$) change in the level of serum total proteins, it significantly increased the serum albumin concentration, as compared to control rats. Administration of either of the extracts to HFD fed rats had no effect on these two parameters (Table 2).

The serum and kidney superoxide dismutase (SOD) activity as well as catalase activity in liver and kidney of untreated HFD fed rats were significantly ($p<0.05$) lower than that recorded in the controls (Tables 3 and 4). High fat diet also caused significant increase in levels of serum TBARS in rats not given either the extract or standard drug. Administration of either of the extracts or the standard drug to HFD rats completely prevented the effects of HFD on serum TBARS as well as organ catalase and SOD activities. The decrease in serum SOD activity caused by HFD was completely prevented by extracts of *O. suave*; but *O. basilicum* extracts caused further significant ($p < 0.05$) decreases.

HFD in rats, with or without administration of basil extracts, caused significant ($p < 0.05$) reductions in the serum levels of urea and creatinine (Table 5), as compared with the controls. There were no statistical differences in these parameters between the HFD rats given the extracts and those not given the extracts. The level of AST in the serum of untreated HFD rats was significantly ($p < 0.05$) higher than that of controls (Table 5). Administration of either of the extracts to HFD rats significantly ($p < 0.05$) lowered the serum AST activity from that recorded in the untreated HFD rats. The serum ALT was not affected by all the treatments.

DISCUSSION

The increases in serum total and LDL cholesterol levels induced by HFD feeding have been reported by earlier researchers (Defronzo et al., 1992). In the present investigation an increase in HDL cholesterol was also induced by the HFD. Diets containing ≥ 40% total calories as lipid or greater than 10% total calories as saturated fat cause hyperlipidemia (Durrington, 1995); in humans however, other factors play various roles

Table 3. Effect of aqueous leaf extracts of *O. basilicum* and *O. suave* on Level of some oxidative stress markers in the serum of rats fed normal or high fat diet.

Parameter	Normal control	Normal + *O. basilicum*	Normal + *O. suave*	HFD control	HF + *O. basilicum*	HFD + *O. suave*	HFD + Lipitor
SOD (u/ml)	434.00 ± 79.06^{a}	51.29 ± 2.19^{b}	22.65 ± 0.50^{c}	73.77 ± 2.99^{d}	54.97 ± 1.92^{b}	477.65 ± 22.84^{a}	72.80 ± 2.94^{d}
Catalase (u/ml) × 10^{-3}	10.70 ± 3.68^{a}	5.50 ± 2.70^{a}	9.40 ± 1.00^{a}	9.30 ± 1.10^{a}	9.00 ± 3.60^{a}	18.30 ± 2.80^{b}	8.60 ± 2.2^{a}
TBARS (MDA) (nmol/ml) × 10^{5}	3.47 ± 0.86^{a}	5.14 ± 1.68^{ac}	8.70 ± 1.35^{a}	6.28 ± 0.89^{cd}	3.83 ± 0.57^{a}	5.05 ± 0.96^{a}	4.77 ± 0.93^{a}

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different ($p < 0.05$)

Table 4. Effect of aqueous leaf extracts of *O. basilicum* and *O. suave* on levels of some oxidative stress markers in the Liver (*) and kidney (**) of rats fed normal or high fat diet.

Parameters	Normal control	Normal + *O. basilicum*	Normal + *O. suave*	HFD control	HF + *O.basilicum*	HFD+ *O. suave*	HFD + Lipitor
Catalase (u/g) x 10^{-2}	*31.00 ± 7.60^{a}	68.00 ± 1.20^{b}	23.60 ± 3.80^{ac}	10.00 ± 5.00^{c}	69.50± 43.00^{b}	42.00 ± 19.50^{a}	66.00 ± 9.80^{b}
	**27.00 ± 18.00^{ab}	52.00 ± 28.00^{a}	17.00 ± 8.00^{b}	19.40 ± 7.00^{b}	29.00 ± 9.00^{b}	19.00 ± 1.80^{b}	72.00 ± 42.00^{c}
SOD (u/g)	*2.54 ± 0.24^{a}	4.37 ± 0.38^{b}	4.65 ± 0.79^{b}	2.80 ± 0.16^{a}	1.17 ± 0.56^{c}	2.68 ± 0.34^{a}	2.65 ± 0.06^{a}
	**7.94 ± 0.08^{a}	4.43 ± 0.23^{b}	6.11 ± 0.61^{c}	2.78 ± 0.23^{d}	13.78 ± 1.53^{e}	2.80 ± 0.27^{d}	2.86 ± 0.17^{d}

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

Table 5. Effect of aqueous leaf extracts of *O. basilicum* and *O. suave* on levels of some markers of renal and hepatic function in the serum of rats fed normal or high fat diet.

Parameters	Normal control	Normal + *O. basilicum*	Normal + *O. suave*	HFD control	HF + *O.basilicum*	HFD + *O. suave*	HFD + Lipitor
Urea (mg/dl)	269.67 ± 27.02^{a}	308.57 ± 8.22^{b}	214.66 ± 8.21^{c}	229.41 ± 22.95^{c}	225.40 ± 22.04^{c}	201.24 ± 20.11^{c}	30.19 ± 10.06^{d}
Creatinine (µmol/l)	102.34 ± 7.72^{a}	32.18 ± 11.16^{b}	155.75 ± 23.45^{c}	81.92 ± 7.45^{d}	92.76 ± 3.47^{d}	86.05 ± 4.80^{d}	70.57 ± 5.65^{e}
AST (u/l)	60.42 ± 9.90^{a}	66.08 ± 6.03^{a}	64.40 ± 8.86^{a}	81.90 ± 6.15^{b}	59.50 ± 8.80^{a}	64.75 ± 10.55^{a}	76.58 ± 7.22^{b}
ALT (u/l)	29.80 ± 2.46^{a}	24.15 ± 2.76^{a}	31.68 ± 5.80^{a}	26.52 ± 1.82^{a}	23.93 ± 2.72^{a}	30.47 ± 4.96^{a}	27.40 ± 2.31^{a}

All values are means ± SD of five replicates. Values with different superscripts along a row are statistically different ($p < 0.05$).

in determining the degree of diet induced hyperlipidemia. The HFD fed animals in the present investigation were fed with 31% lipid content contrasted with the 7% lipid content of control diet. Serum triacylglycerols levels were lower in the HFD rats than in the controls. Administration of either of the *Ocimum* extracts to HFD rats significantly reversed the diet induced dyslipidemia; though not to the same extent to which the standard hypolipidemic drug (Lipitor®) did.

Lipid lowering drugs in the market fall into various categories (Rucker et al., 2007); including, those that inhibit HMG CoA reductase, called Statins (of which Lipitor® is an example); those that sequester bile acids (Resins), and hence increase the excretion of bile acids and salts (thence, cholesterol); those that reduce intestinal fat

absorption and those that activate lipoprotein lipase (Fibric acid derivatives). The active principle(s) in the extracts used in this work may have acted in one or more of these ways.

Diet induced hyperlipidemia increased serum TBARS and decreased serum and organ catalase and SOD activities; all indicative of increased oxidative stress. Hyperlipidemia has been reported to increase production of reactive oxygen species (ROS) by polymorphonuclear leukocytes and monocytes (Wilson and Gelb, 2002). These ROS cause damage to subcellular structures including membrane components, DNA and certain proteins (Flora, 2007). This undoubtedly contributes to the etiology of diseases associated with hyperlipidemia, such as cardiovascular diseases and type 2 diabetes mellitus. Administration of the extracts completely prevented the diet induced increase in serum TBARS, but only partially, though significantly, prevented the decreases in serum and organ catalase and SOD activities caused by HFD.

Ocimum species has been extensively reported for its essential oil content (Roberto et al., 2003); however, the antioxidant capacity of the plant extracts is mainly dependent on phenolic compounds (Ramarathnam et al., 1997).

The significant reduction in serum urea and creatinine observed in untreated HFD rats may be attributed to the relatively lower protein content of the HFD (11%) as compared to the normal feed (15%). Administration of either of the extracts had no effect on the serum urea and creatinine in the HFD rats. AST activity was significantly elevated by HFD, indicating organ damage. This damage can reasonably be attributed to the increased oxidative stress induced by HFD. The diet induced increase in AST was prevented by administration of the basil extracts; and this may be tied to the lowering of oxidative stress by the extracts as recorded in the HFD fed animals; since increased free radical load causes tissue damage.

In conclusion, HFD induced hyperlipidemia and oxidative damage can be prevented by aqueous extracts of *O. suave* and *O. basilicum*.

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