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Effect of *Ficus capensis* chloroform extract and *Eucalyptus camaldulensis n*-hexane extract on blood glucose and lipids in normal Wistar rats

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Abstract
The present study was designed to investigate the effects of the stem bark and leaf extracts of *Eucalyptus camaldulensis* and *Ficus* capensis on the blood glucose and lipid profile in normal albino rats. Forty adult albino rats were classified into two sets (blood glucose and lipid profile determination) with five groups comprising of four animals per group. The first four groups (treatment groups) in each set were administered 100, 400, 800 and 1200 mg/kg body weight of each extract, the fifth group (control group) received 5 ml of 0.90% normal saline solution for a 7 days duration. The result showed a decrease in blood glucose concentration in a dose dependent manner but was however seen to increase at 1200 mg/kg bw when the chloroform extract of *F. capensis* was administered. The n-hexane extract of *E. camaldulensis* caused a corresponding increase in blood glucose concentration in a dose dependent manner. In both determinations and in comparison, to the control group, there were statistically significant differences between the treatment groups at 95% confidence level for all lipid parameters determined. The findings of this study indicate that while the chloroform extract of *F. capensis* possesses anti-diabetic property, the n – hexane extract of *E. camaldulensis* does not have anti-diabetic effect, but rather appears to promote bioavailability of glucose in the blood. Both extracts however showed promising anti-lipidemic activity.

Keywords: *Eucalyptus camaldulensis; Ficus capensis;* Glucose; Lipids

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. In Nigeria, almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession [1]. It is estimated that about 80% of the world population relies on botanical preparations as medicines to meet their health needs [2]. Herbal medicine is readily available in our diverse vegetation, cheap and above all carries the potential for introducing new templates into modern medicine [3]. In the Igala speaking areas of Nigeria, herbal medicine practitioners are still consulted as a first choice in the treatment of wide range of ailments, because traditional medicine blends readily with the socio-cultural life of the people, and the fact that orthodox medicine are more expensive to procure. In addition, orthodox pharmaceutical preparations are many times faked [4].

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Ficus capensis belongs to the mulberry family Moraceae. It is known as "*uwaryara"* in Hausa, "o*poto"* in Yoruba, "*rima bichehi"* in Fulani, "*akokoro"* in Igbo and "*obada"* in Edo [5-6]. The plant possesses spreading roots, branches and broad green leaves. Traditionally the plant is used in the treatment of dysentery, oedema, epilepsy, chest ailments, leprosy, tuberculosis, anemia, rickets in infants among others [6, 7-9]. Scientifically, the plant has been reported to possess anti-sickling [10-11], antibacterial [8], antiabortifacient [12], immune-stimulatory [11], antidiarrhoeal [12], antioxidant [13] and pro-fertility in treating azoospermia [14]. The plant, *Eucalyptus camaldulensis*, commonly known as the "*river red gum*", belongs to the family of Myrtaceae. It is a large evergreen plant, 20 - 40m high, with a stout trunk, widely spreading, irregular smooth bark shedding at intervals throughout the year to show white, yellow and grey, becoming rough at the base [4]. The Igala people call it "*ogwuiba*" meaning fever remedy, and it is used for the treatment of a wide range of ailments.

Bearing in mind that identifying and isolating the effective extract/ fractions of a crude extract may prove better therapeutically and probably less toxic than its crude form, this present study aims at evaluating the glycemic and lipid profile of chloroform leaf extract of *F. capensis* and n-hexane stem bark extract of *E. camaldulensis* on albino rats sub-chronically treated for 7 days.

EXPERIMENTAL

Plants collection and authentication. The plant materials were collected from Anyigba town in North-Central Nigeria. They were identified by Prof. COC Agwu of the Biological Sciences Department, Kogi State University, Anyigba, Nigeria. They were collected in bags prior to them being washed with running tap water to remove earthy impurities. They were shade dried at room temperature for two weeks before being pulverised using high-speed Creston mechanical grinder. The pulverised samples were stored in airtight glass container until ready to use.

Experimental animals. Forty healthy albino rats (*Rattus novergicus)* of either sex with an average weight of 180 ± 5 g were obtained from the Animal House of the Department of Biochemistry, Kogi State University, Anyigba. The animals were housed in wooden cages and had free access to standard rat pellets and water *ad libitum*. They were randomly divided into 5 groups of 4 rats each, maintained under laboratory conditions (temperature 24-28°C, relative humidity 60-70% and 12 h light-dark cycle). Rats were housed at the Animal House of the Department of Biochemistry, Kogi State University, Anyigba before administration of extract. Rats in groups 1, 2, 3 and 4 were administered 100, 400, 800 and 1200 mg/kg bw p.o. of the extracts respectively daily for 7 days using an oral intubator. Rats in group 5 received 5 ml of 0.90% normal saline in lieu of the extract for the same period Permission was granted by the Research Ethics Committee of Kogi State University, Anyigba before embarking on the animal studies.

Extracts preparation

N-hexane extract of *Eucalyptus camaldulensis* (NEEC) and chloroform extract of *Ficus capensis* (CEFC). One thousand grams of the pulverised stem bark of *Eucalyptus camaldulensis* was cold macerated in five litres of n-hexane in a capped vessel for 24 hours. The macerate was filtered through Whatman No 1 filter paper using a Speedvac vacuum pump. The filtrate was then concentrated using a rotary evaporator and then air dried to obtain the n-hexane extract of *Eucalyptus camaldulensis* (NEEC). The same starting quantity and procedure was followed using the pulverized leaf of *Ficus capensis* to obtain the chloroform extract of *Ficus capensis* (CEFC). The percentage yields were

determined relative to the starting material. The extracts were kept in airtight glass containers and refrigerated at -4^{o}C until use.

Blood glucose concentration determination. On the eighth day after the 7 days extracts treatment, drops of blood were obtained from the tail of the animal. The blood was applied on the disposable fine test strip of the Roche Accu-Chek Glucometer. The blood glucose concentration was then determined and recorded. The procedure was repeated for all animals in each group.

Preparation of serum and lipid profile determination. Serum was prepared by aspiration of the clear yellowish liquid after clotting and centrifuged for 5 minutes at 1000g in a Uniscope SM 112 bench centrifuge. The clear supernatant was used for the estimation of serum lipids (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) according to the method described by Bukolo and David [15] using reagent kit obtained from Randox Laboratories Limited. The procedure was repeated for all animals in each group.

Statistical analysis. Results obtained were presented as mean ± SD of four observations. Variation within a set of data was analysed by one-way analysis of variance (ANOVA) using the Graph Pad Prism Software (GPPS). Values of $p<0.05$ were taken as statistically significant.

RESULTS AND DISCUSSION

Extraction. The crude extracts of n-hexane of the stem bark of *Eucalyptus camaldulensis* and chloroform of the leaf of *Ficus capensis* gave a percentage yield of 12.57% and 15.26% respectively.

Effect of NEEC and CEFC on Blood Glucose Concentration. The mean effect of NEEC and CEFC on the concentration of blood glucose of the treated animals compared to the control is presented in Table 1. Tables 2 (a) and (b) show the mean values of the lipid profile of the treatment group relative to the control for *F. capensis* and *E. camaldulensis* respectively. The result presented in table 1 showed a decrease in blood glucose concentration except at the dose of 1200 mg/kg bw when the CEFC was administered. This was not the case with the NEEC, which cause a corresponding increase in blood glucose concentration in a dose dependent manner. The increase in blood glucose level was however within the normal range of 50-160 mg/dl for albino rats [16]. This result suggests that while the CEFC extract may have an anti-diabetic property, the NEEC extract does not have anti-diabetic effect, but rather appears to promote bioavailability of glucose in the blood. The blood glucose concentration for both extracts was found to be statistically significant when compared with the control group. The blood glucose lowering activity of *F. capensis* may be due to the presence of phytosterols in the chloroform extract as reported by Musa *et al*., [4]. Similarly, the lipids lowering effect (Table 2a) caused by the CEFC is in agreement with results obtained by Das *et al*., [17]. This result is significant because increased plasma cholesterol, particularly LDL is one of the most important risk factors for coronary vascular disease. LDL particle are taken up by macrophage cells after oxidized or modified and then deposited in the arterial intima leading to formation of atheroma [18]. The lowering HDL levels however is not considered as ideal because it poses a strong risk factor for coronary heart disease as HDL act as antioxidant and protect LDL from oxidation so as to reduce LDL concentration from circulation [19 -20]. The lipid lowering effect could also be attributed to the tannin, phenols and flavonoids as reported by Musa *et al*. [21]. Tannin and flavonoids have been reported to have antioxidant activity hence play a role in preventing coronary heart disease among others hence a reduction in the major risk

factor (lipids) [22]. The result of the NEEC showed a significant reduction in the entire lipid investigated except for HDL, which increases in a dose dependent manner. This result demonstrated the ability of NEEC to influence liver metabolism towards reduced synthesis of lipids. While an increase of 1% serum cholesterol notably HDL is reported to result in a 3% decrease in coronary heart disease [23], a reduction in LDL by 2 mg/dl can result in 1% increase in the risk for coronary artery disease [24]. The significant reduction in the serum lipid by NEEC may be due to suppression of LDL oxidation [25].

Table 1: Effect of NEEC and CEFC on blood glucose concentration in normal albino rats

Treatment Groups	Administered Dose	Blood Glucose Concentration (mg/dl)	
		CEFC	NEEC
1	100	97 ± 5.21^{a}	82 ± 5.15^{b}
2	400	95 ± 4.43^{a}	92 ± 4.02^{b}
3	800	83 ± 3.22^{a}	98 ± 3.52^{b}
4	1200	99 ± 2.25^{a}	104 ± 2.51^{b}
5	5 ml of 0.90% normal saline	76 ± 2.91^{b}	79 ± 5.35^{c}

CEFE = Chloroform extract of *Ficus capensis*. NEEC = n-hexane extract of *Eucalyptus camaldulensis*. Values are Mean ± SD (n=4). Values with different alphabet superscript in a column are significantly different at $P < 0.05$.

Table 2(a): The Mean values of lipid profile of experimental rat groups administered with *F. capensis.*

Treatment Groups	Administered dose	Lipid Concentration (mg/dl)				
		TC	TG	HDL	LDL	HDL/LDL
1	100	60.76 ± 4.23^{a}	139.60 ± 4.56^{c}	23.62 ± 4.21^{e}	9.22 ± 4.44^{g}	2.56
2	400	58.30 ± 3.23^{a}	129.23 ± 3.11^{c}	23.36 ± 3.10^{e}	9.09 ± 3.41^{g}	2.57
3	800	53.56 ± 3.87^{a}	125.23 ± 3.31^{c}	22.26 ± 3.54^{e}	6.25 ± 3.67^{g}	3.56
4	1200	48.35 ± 4.23^{a}	114.18 ± 4.44^{c}	17.26 ± 4.12^{e}	2.25 ± 4.87^{g}	7.67
5	5 ml of 0.9% normal saline	122.24 ± 2.12^{b}	149.57 ± 3.24^{d}	40.42 ± 4.21^{f}	61.91 ± 3.66^{h}	0.49

TC = Total Cholesterol; TG = Triglycerides; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein. Values are Mean ± SD (n=4). Different alphabetical superscript along a column significantly different ($P < 0.05$)

Table 2(b): The Mean values of lipid profile of experimental rat groups administered with *E. camaldulensis*

Treatment Groups	Administered Dose	Lipid Concentration (mg/dl)				
		TC	TG	HDL	LDL	HDL/LDL
1	100	115.79 ± 1.12^{a}	148.98 ± 1.43^{c}	34.02 ± 1.22^{e}	51.97 ± 1.33^{g}	0.65
2	400	105.79 ± 4.23^{a}	127.97 ± 4.10^{c}	36.18 ± 4.78^{e}	44.02 ± 4.41^{g}	0.82
3	800	98.64 ± 3.12^{a}	123.01 ± 3.21^{c}	48.09 ± 3.41^{e}	25.95 ± 3.63^{g}	1.85
4	1200	95.76 ± 1.22^{a}	113.01 ± 1.32^{c}	62.16 ± 1.26^{e}	11.00 ± 1.75^{g}	5.65
5	5 ml of 0.90% normal saline	121.34 ± 2.11^{b}	146.45 ± 2.31^{d}	33.24 ± 2.44^{f}	59.99 ± 2.13^{h}	0.55

TC = Total Cholesterol; TG = Triglycerides; HDL = High Density Lipoprotein; LDL = Low Density Lipoprotein. Values are Mean ± SD (n=4). Different alphabetical superscript along column are significantly different ($P < 0.05$)

The elevation of HDL - cholesterol might suggest that the NEEC contain ingredients capable of enhancing the activities of hepatic lipogenic and cholesterogenic enzymes, such as malic enzyme, fatty acid synthase, glucose 6-phosphate dehydrogenase and HMG-CoA reductase, which are all required for cholesterol synthesis [23]. Both extracts improved the HDL/LDL ratio significantly ($p<0.05$) in a concentration dependent manner hence could be inferred to promote cardiovascular health.

Conclusion. The study reports that the leaf extract of *Ficus capensis* and the stem bark of extract of *Eucalyptus camaldulensis* possess phytochemicals with positive effects on lipid profile in rats hence could be used as an antiatherogenic agent for the management of

atherosclerosis in man. This validates its traditional uses in the management of cardiovascular diseases arising from dyslipidaemia.

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