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Cholesterol lowering effects of *Acacia nilotica subalata* in Normal and Type 2 Diabetic Male Rats

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ABSTRACT

OBJECTIVE: To investigate the effects of *Acacia nilotica subalata* on plasma lipids in normal and type 2 diabetic male rats.
METHODS: Diabetes was induced in 18 out of 30 rats. The rats were in five groups: Group A normal control, Group B diabetic control, Group C diabetic rats treated with *Acacia nilotica subalata*, Group D normal rats treated with *Acacia nilotica subalata* and Group E diabetic rats receiving metformin. Blood glucose was measured with a glucometer. Lipids were assayed by colorimetric methods. Data were expressed as mean ± standard error of mean and analyzed using analysis of variance. Results were considered statistically significant if $p < 0.05$.
RESULTS: *Acacia nilotica subalata* and metformin decreased total cholesterol in Groups C and E compared to diabetic control Group B (109.05 ± 9.134 and 90.69 ± 6.838 vs 153.89 ± 18. 829 mg/dl, $p < 0.05$). *Acacia n. subalata* and metformin in Groups C and E significantly decreased low density lipoproteins (LDL) compared to diabetic control Group B (59.62 ± 6.532 and 42.32 ± 4.844 vs 105.56 ± 15.14 mg/dl, $p < 0.05$). *Acacia nilotica subalata* leaf extract may be considered beneficial against hyperlipidemia induced by diabetes mellitus.

Keywords: *Acacia nilotica subalata*, lipids, diabetes

INTRODUCTION

Diabetes mellitus is a syndrome characterized by persistent hyperglycemia and other abnormalities of carbohydrate, fat and protein metabolism, resulting from defective insulin secretion by the pancreas, insulin action, or sometimes both [15]. Diabetes mellitus requires lifelong- term treatment which is very hard for diabetic patients to support. A large number of synthetic antidiabetic drugs are available that reduce the effects of diabetes mellitus and its related complications, but no cure is available yet. In addition, diabetic patients suffer adverse effects associated with various synthetic antidiabetic drugs. Due to various challenges in management of diabetes mellitus using synthetic drugs, the herbal products are gaining popularity in developing and developed countries because they are believed to have lesser side effects, low cost, and easier accessibility [18]. Several plant products have been used as potential therapeutic agents in the management of diabetes mellitus. The Acacia has been reported to have cholesterol-lowering and hypoglycemic effects, although there is insufficient evidence in support of those observations [2].

Acacia is a genus with many species.

Acacia nilotica subalata is one of subspecies found in Kenya. The Acacia nilotica has been used in traditional medicine in many situations such as treatment of diarrhea, leprosy, asthma, cancers of eye and tuberculosis [21]. Acacia nilotica was considered as remedy that is helpful for treating premature ejaculation [20] Extract of Acacia nilotica has shown analgesic and antipyretic properties [8]. *Acacia nilotica nilotica* has been reported to have antihypertensive and antispasmodic effects [12]. *Acacia nilotica indica* leaves are rich in polyphenols known to lower blood glucose and tannins [4] which antagonize calcium-induced contraction of smooth and heart muscle, thereby reducing blood pressure [12].

Diabetes mellitus causes impairment of control of blood glucose and high level of cholesterol. The aim of this study was to demonstrate the effects of *Acacia nilotica subalata* on plasma

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lipids in normal and type 2 diabetic male rats.

METHODS

Collection of *Acacia nilotica subalata* leaves: Before collection of *Acacia nilotica subalata* leaves, identification of the plant was done by Botany Department of University of Nairobi. Acacia *nilotica subalata* leaves were collected in February 2012 in the Athi river area of Machakos county, Kenya.

Extraction: *Acacia nilotica subalata* leaves were washed free of debris and dust particles and air dried at room temperature for three days. The leaves were ground using electrical grinder (Wiley Mill, model 2, Arthur H. Thomas company, Philadelphia, USA). The product obtained was mixed with 97% ethanol and soaked for two days. Then it was filtered using cotton inserted in the filter funnel. The concentration of the filtrate was achieved using a rotary evaporator (ROVA-2L, mrc).

Experimental animals: 30 healthy male Wistar rats aged about 6 to 8 months, weighing 200 - 350g, from the Department of Zoology, Kenyatta University and the Department of Biochemistry, University of Nairobi, were housed in the animal house of Department of Medical Physiology, University of Nairobi. Rats were marked and each group assigned to a cage. The animals were allowed to acclimatize to standard laboratory conditions of room temperature (25 ± 2 º C) and a 12 h light-12h dark cycle. They received standard rat feed (mice pencils supplied by Unga Farm care, Ltd) and free access to water.

Induction of experimental type II Diabetes Mellitus and experimental design: Before induction the rats were fasted for 16- 18 hours. Diabetes mellitus was induced in 18 outof 30 rats by injecting intraperitoneally 150 mg/kg body weight alloxan (4% weight/ volume) [24]. The confirmatory test of diabetic rats was fasting blood glucose level > 7 mmol/l measured after one week [23]. A second dose of alloxan 100 mg/kg body weight was administered to animals that were not diabetic in the first instance.

The rats were assigned into five groups as follows: Group A normal control received normal saline, Group B diabetic control received normal saline, Group C diabetic rats treated with *Acacia nilotica subalata* extract, Group D normal rats received *Acacia nilotica subalata* extract and Group E diabetic rats treated with metformin. Each group had six rats.

Groups B, C, and E received intraperitoneally (150 mg/kg body weight) alloxan 4% w/v dissolved in 20 ml of normal saline. The same volume of normal saline was injected to Groups A and D. Each morning the animals of Groups C and D received orally (800 mg/kg body weight) *acacia nilotica subalata* leaf extract dissolved in 30 ml of normal saline. Group E received orally (100 mg /kg body weight) metformin (M- FORLIN 500, LINCOLN Pharmaceuticals LTD, Gujarat, India) dissolved in 10ml of normal saline. The duration of treatment was 6 weeks.

In the morning, by tail amputation using a tail snip, blood for glucose measurement was collected. Once a week blood glucose was measured by glucometer (On Call Plus, ACON Laboratories Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121, USA). At the end of the experiment, the animals were anesthetized with inhalation of diethyl ether 0.706g/l at 25%. Blood samples (2 ml) of each rat in all groups drawn by cardiac puncture and taken in ethylene -diamine-tetraacetic acid (EDTA) tubes were analyzed for lipids.

Total cholesterol was assayed by colorimetric methods using commercial kits (Enzymax, Vitro Scient, Egypt). This method involved enzymatic reactions. The intensity of the color produced was directly proportional to cholesterol concentration. It was determined by measuring the increase in absorbance at 545 nm by Humalyzer 2000 (Human, SI. 2500 -3723, Germany).

The High Density Lipoprotein (HDL) cholesterol was measured by enzymatic colorimetric test (cholesterol liquicolor test, Human campany, Germany). It was determined by measuring the increase of absorbance at 546 nm (Humalyzer 2000, Human, SI. 2500 -3723, Germany). The Low Density Lipoprotein (LDL) levels were calculated using the formula: LDL= total cholesterol – HDL– (triglyceride/5) [11]. Triglycerides were measured by enzymatic colorimetric test (triglycerides test kit, Vitro Scient, Egypt). It was determined by measuring increase in absorbance at 545 nm (Humalyzer 2000, Human, SI. 2500 -3723, Germany).

Ethical consideration: This study put in consideration the guidelines for care and use of laboratory animals as established by the Federation of the European Laboratory Animal Science Association (FELASA), the European Society of Laboratory animal Veterinarians (ESLAV) and the European College of Laboratory Animal Medicine (ECLAM) [22].

Data analysis: Data was analyzed on SPSS version 16.0. The data was presented as mean ± S. E.M (Standard Error of Mean) and analyzed using Analysis of Variance (ANOVA) with multiple comparisons versus control groups by Tukey's method. Results were considered as statistically significant if $p < 0.05$.

RESULTS

Fasting blood glucose profile: Before treatment (day 0), in diabetic groups (B, C and E) blood glucose levels were significantly elevated compared to the normal control Group A (15.54± 0.580, 16.28 ± 3.321 and 14.93 ± 2.31 vs 4.47 ± 0.114 mmol/l, $p < 0.05$) respectively. *A. n. subalata* extract significantly decreased blood glucose levels in diabetic Group C compared to diabetic control Group B (7.08 ± 1.451 vs 18.10 ± 1.378 mmol/l, $p < 0.05$). Treatment with *A. n. subalata* extract (Group C) and metformin (Group E) showed no significant difference in reduction of blood glucose (7.08 ± 1.451 vs 6.50 ± 1. 10 mmol/l, $p = 0.992$). There was no significant difference in blood glucose between normal Group D treated with with A. n. subalata extract and normal control Group A (4.52 ± 0.188 vs 4.53 ± 0.185 mmol/l, $p = 1$).

Table 1: Effect of Acacia niloticasubalata on fasting blood glucose

Groups of rats	Day0 Mean± SEM	Day14 Mean ±SEM	Day28 Mean ±SEM	Day35 Mean± SEM	Day42 Mean± SEM
A	4.47 ± 0.11	4.33 ± 0.16	4.53 ± 0.20	4.37 ± 0.14	4.53 ± 0.18
D	4.48 ± 0.24	4.50 ± 0.26	4.48 ± 0.08	4.66 ± 0.43	4.52 ± 0.18
B	15.54 ± 0.58 ab	17.27±1.33	18.48 ± 1.69	18.76 ± 1.37	18.10 ± 1.37
C	16.28 ± 3.32 ac	9.32 ± 0.27 bc	8.22 ± 2.17bc	7.96 ± 1.42 bc	7.08 ± 1.45 bc
E	14.93 ± 2.31 ae	7.68 ± 1.78 be	6.38 ± 0.78 be	6.32 ± 1.06 be	6.50 ± 1.10 be

Group A: normal control, Group B: diabetic control, Group C: diabetic treated with plant Extract, Group D: normal group treated with plant Extract, Group E: diabetic rats treated with metformin., ab: p < 0.05 group B as compared to group A. ac : p < 0.05 group C as compared to group A. ae: p < 0.05 group E as compared to group A. bc: p < 0.05 group C as compared to group B. be : p < 0.05 group E as compared to group B.

Plasma lipid profile: There was a statistically significant elevation of total plasma cholesterol in diabetic control Group B compared to normal control Group A (153.89 ± 18.829 vs 98.70 ± 2.643 mg/dl, p < 0.05). The administration of *A. n. subalata* extract and metformin statistically decreased total plasma cholesterol (Groups C and E) compared to diabetic control Group B (109.09 ± 9.131 and 90.69 ± 6.838 vs 153.89 ± 18.829 mg/dl, p < 0.05) respectively. There was no statistically significant difference between diabetic rats (Group C) treated with *A. n. subalata* and those treated with metformin (Group E) (p = 0.738). When the comparison was done between normal Group D treated with *A. n. subalata* extract and normal control Group A there was no statistical difference in total plasma cholesterol levels (100.05 ± 9.930 vs 98.70 ± 2.643 mg/dl, p = 1).

Diabetic control Group B had a statistically significant decrease of HDL cholesterol compared to the normal control Group A (23.47 ± 2.645 vs 36.08 ± 2.343 mg/dl, p < 0.05). A statistically significant elevation of HDL cholesterol was shown in Group E (treated with metformin) compared to diabetic control Group B (34.66 ± 4.004 vs 23.47 ± 2.645 mg/dl, p < 0.05), but not in Group C (treated with *A. n. subalata* extract) compared to diabetic control Group B (30.05 ± 1.976 vs 23.47 ± 2.645 mg/dl, p = 0.408).

Reduction of triglycerides levels in Groups C and E, respectively treated with *A. n. subalata* extract and metformin was not statistically significant compared to diabetic control Group B (96.10 ± 35.764 and 74.59 ± 16.765 vs 128.02 ± 17.837 mg/dl, p > 0.05).

The LDL cholesterol level was statistically increased in diabetic control Group B compared to the normal control Group A (105.56 ± 15.14 vs 45.15 ± 4.198 mg/dl, p < 0.05). Treatment with either *A. n. subalata* extract or metformin in Groups C and E, significantly decreased LDL cholesterol levels respectively compared to diabetic control Group B (59.62 ± 6.532 and 42.32 ± 4.844 vs 105.56 ± 15.14 mg/dl, p < 0.05).

Table 2: Effect of Acacia niloticasubalata on plasma lipids

		Total cholesterol Mean±SEM	HDL Mean± SEM	Triglycerides Mean± SEM	LDL Mean± SEM
group	A	98.70 ± 2.64	36.08 ± 2.34	97.39 ± 17.79	43.15 ± 4.19
	D	100.05 ± 9.93	38.17 ± 0.69	70.12±18.70	55.79 ± 17.26
	B	153.89 ± 18.82 ab	23.47 ± 2.64ab	128.02 ± 17.83	105.56± 15.14ab
	C	109.09 ± 9.13 bc	30.05 ± 1.97	96.10 ± 35.76	59.62 ± 6.53 bc
	E	90.69 ± 6.83 be	34.66 ± 4.00 be	74.59±16.76	42.32 ± 4.84 be

A: Normal control, B: Diabetic control, C: diabetic treated with plant extract, D: Normal group treated with plant extract, E: Diabetic group treated with metformin. .,ab: p < 0.05 group B as compared to group A. bc: p < 0.05 group C as compared to group B. be : p < 0.05 group E as compared to group B.

DISCUSSION

Fasting blood glucose: This study showed hypoglycemic activity of *Acacia nilotica subalata* leaf extract in diabetic rats. The results are similar to those of Maqsood et al. (2008) who reported the glucose- lowering effect of *Acacia nilotica nilotica* in diabetic rabbits [16]. The hypoglycemic activity of *Acacia nilotica subalata* extract possibly occurs by stimulating the activity of the β cells of the pancreas and/or due to its insulin-like action on insulin –sensitive cells. This suggests that *Acacia nilotica subalata* leaf extract at the dose of 800 mg/kg body weight, may induce β cell regeneration. The exact mechanism for this action remains unclear. The antihyperglycemic effect of *Acacia nilotica subalata* may be the result of the action of the polyphenols found in the plant extract [17].

Polyphenols have been shown to act as scavengers for oxygen and nitrogen-free radicals, protecting the fatty membranes of cells, proteins and DNA [13], a property that could stop damage of the remaining β cells. In addition the polyphenols may help regenerate activity of β cells. polyphenols and tannins have the ability to bind with digestive enzymes and inhibit the activities of α-amylase and α-glucosidase in the gut [19], therefore reducing the glucose absorption from small intestine.

The normal values of blood glucose found in normal Group D may be due to the normal homeostasis of glucose in normal animals, which acts through negative feedback systems to maintain blood glucose levels within the normal range [14].
The decrease of blood glucose in Group E that was treated with metformin results from the mechanism of action of metformin which acts by suppressing glucose production by the liver [25]. In addition, metformin increases sensitivity to insulin, enhances peripheral glucose utilization and decreases glucose absorption from small intestine [7].

Plasma lipid profile: In the present study the levels of LDL, triglycerides and total cholesterol were elevated in diabetic Group B whereas plasma HDL level decreased. These results concur with those of Alarcon-Aguilar et al. (2002) in diabetic rats and mice [1]. The higher lipid levels found in diabetic rats were due to increased mobilization of free fatty acids from peripheral deposits and also to lipolysis caused by hormones [14].

The present study showed a significant reduction of total cholesterol, LDL in diabetic Groups C and E, and a significant increase of HDL levels in Group E treated with metformin, but the reduction of triglycerides levels is not significant. A number of other plant extracts have been reported to have hypoglycemic and hypolipidemic and insulin stimulatory effects [10]. In this regard the *Acacia nilotica subalata* extract possibly causes regeneration of β cells of the pancreas leading to increase of insulin secretion. The increase in insulin secretion consequently decreases blood glucose level which may lead to inhibition of lipid peroxidation and control of lipolytic hormones. The beneficial effects of *Acacia nilotica subalata* on lipid profile in induced diabetic rats may be secondary to better glycemic control.

Metformin causes beneficial effects on lipid profile by correcting abnormal glucose metabolism [9]. It also moderately decreases triglycerides levels as a result of decreased hepatic synthesis of very-low-density lipoprotein [5]. Metformin appears more beneficial in preventing cardiovascular complications associated with Type 2 Diabetes Mellitus than *A. n. subalata* extract because it increased HDL, decreased LDL and triglycerides [3].

CONCLUSION

The present study showed that ethanolic leaf extract of *A. n. subalata* produced hypoglycemic effect in alloxan-induced diabetic male rats, at the dose of 800mg/kg. It significantly decreased total cholesterol, LDL cholesterol, but the increase of HDL cholesterol was not statistically significant. Consequently *Acacia nilotica subalata* leaf extract is a herbal product with potential to decrease blood glucose and hyperlipidemia in type 2 diabetes mellitus. This study investigated parameters which are common in diabetes. Since the use of plant extract may change other plasma parameters, further studies are required to investigate the detailed mechanism of action of *Acacia nilotica subalata* leaf extract on cholesterol –lowering activity and its effects on other metabolites.

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