BODY WEIGHT LOWERING EFFECT OF *ACACIA NILOTICA SUBALATA* IN NORMAL AND TYPE 2 DIABETIC MALE RATS

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ABSTRACT

Objectives: to investigate the effects of *Acacia nilotica subalata* on body weight 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 received *Acacia nilotica subalata*, group D normal rats received *Acacia nilotica subalata* and group E diabetic rats received metformin. Blood glucose was measured by glucometer. Data were presented as mean ± standard error of mean and analy analysis of variance performed. Results were considered statistically significant if p value < 0.05.

Results: Treatment with *A. n. subalata* extract significantly decreased blood glucose in diabetic group C compared to diabetic control group B (7.08 ± 1.451 vs 18.10 ± 1.378 mmol/l, p < 0.05). Diabetic control group B lost 6.6 % of their initial body mass. Diabetic group C (treated with *A. n. subalata* extract) and E (treated with metformin) respectively lost 9. 54 % and 11.29%. Normal group D treated with *A. n. subalata* lost 0.30% of its initial body mass. *Acacia nilotica subalata* leaf extract has the potential to combat obesity and lower blood glucose in normal and type 2 diabetes mellitus.

Keywords: Acacia nilotica subalata, weight, diabetes

INTRODUCTION

The use of medicinal plants to treat diseases can be traced back over five millennia to written documents of the early civilization in China, India and the Near East. The ancient Greeks and Romans used medicinal plants.

Greek physicians wrote the first European treatise on the properties and uses of medicinal plants, the Materia Medica [24]. These days people of all continents use indigenous plants for treatment of various diseases. One such disease is diabetes mellitus which leads to abnormalities in carbohydrate, protein and lipid metabolism. It is one of the most common diseases with a worldwide prevalence estimated to be over 6 per cent of the world population [7]. Several herbal products have been used as potential therapeutic agents in the management of diabetes mellitus and its related complications. One such product is *Acacia.* This plant has been reported to have cholesterol-lowering and antidiabetic effects, although there is insufficient evidence in support of those observations [2]. Acacia is a genus with more than 1350 species.
Acacia nilotica subalata is one of subspecies found in Kenya. Aqueous extract of Acacia nilotica (A.n) pods, fruits, bark and seeds have traditionally been used to treat diarrhea, leprosy, asthma, skin diseases, cancers of eye and ear, tuberculosis and small pox [19]. Extract of Acacia nilotica has shown analgesic and antipyretic activities [6] and ability to block platelet aggregation [8]. Aqueous extracts of fruits and stem bark showed molluscicidal activity [10]. *Acacia nilotica indica* leaves are rich in tannins and polyphenols [3], known to decrease blood glucose levels [17]. Some phenolic compounds with dihydroxyl groups can form complexes with transition metals, preventing metal- induced free radical formation. Tannins bind to proteins in the gastrointestinal pathway and subsequently decrease feed intake. In addition they have the ability to bind and inhibit the digestive enzyme effects [12]. In this regard the aim of this study was to demonstrate the effects of *Acacia nilotica subalata* on body weight in normal and type 2 diabetic male rats.

METHODS

Collection of Acacia nilotica subalata leaves

Acacia nilotica subalata leaves were collected in February 2012 from Athi river area, along Mombasa road in Machakos county of Kenya. Identification was done by Botany Department, University of Nairobi.

Extraction

Acacia nilotica subalata leaves were washed free of debris 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 ground product was mixed with 97% ethanol and soaked for two days. Thereafter it was filtered using cotton. The filtrate was concentrated using rotary evaporator (ROVA-2L, mrc).

Experimental animals

Thirty healthy male Wistar rats of about 6 to 8 months, weighing 200 - 350g, living in the same conditions were procured from the Department of Zoology, Kenyata University and the Department of Biochemistry, University

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of Nairobi. The rats were housed in the animal house of Department of Medical Physiology, University of Nairobi. Individual animals were marked and each group assigned to a cage. The animals were allowed to acclimatize to laboratory conditions for one week. Standard laboratory conditions of room temperature (25 ± 2 º C) and a 12 h light- 12h dark cycle were maintained. The rats received standard rat feed (mice pencils supplied by Unga Farm care Ltd) and had unlimited access to water. Each morning the cages were cleaned.

Induction of experimental type 2 diabetes mellitus and experimental design

The animals were fasted for 16- 18 hours with free access to water. Diabetes mellitus was induced in 18 out of 30 rats by administering 150 mg/kg body weight alloxan (4% weight/ volume) [22]. Diabetes was confirmed by measuring fasting blood glucose level > 7 mmol/l after one week [21]. A repeat dose of alloxan 100 mg/kg was injected to animals that failed to acquire diabetes 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 received *Acacia nilotica subalata* extract, group D normal rats received *Acacia nilotica subalata* extract and group E diabetic rats received metformin. Each group contains six rats. Groups (B, C, and E) received intraperitoneally (150 mg/kg body weight) alloxan 4% w/v prepared with 20 ml of normal saline. The same volume of normal saline was administered to group A and D. Each morning the rats of group C, 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 course of treatment in each group was 6 weeks. Blood for glucose measurements was collected from the study rats by tail amputation using a tail snip. Once a week blood glucose was measured by glucometer

(On Call Plus, ACON Laboratories Inc. 4108 Sorrento Valley Boulevard, San Diego, CA 92121, USA). The weight was measured with the balance (Triple beam balance,US. Pat. Nº 2.729.439, Ohaus scale corporation, Florham Park. N.J. USA) at the beginning, every two weeks, until the end of experiment.

Data analysis

Data were analyzed on SPSS version 16. The data were expressed as mean ± S. E.M (Standard Error of Mean) and statistically analyzed using Analysis of Variance (ANOVA) with multiple comparisons versus control groups by Tukey's method. Results were considered as statistically significant if p value < 0.05.

RESULTS

Fasting blood glucose profile

Fasting blood glucose in diabetic groups B, C and E before treatment (day 0) were significantly high 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. Treatment with A. n. subalata extract significantly decreased blood glucose in diabetic group C compared to diabetic control group B (7.08 ± 1.451 vs 18.10 ± 1.378 mmol/l, $p < 0.05$). Blood glucose reduction was not statistically different between groups treated with A. n. subalata extract (group C) and metformin (group E) (7.08 ± 1.451 vs 6.50 ± 1. 10 mmol/l, $p = 0.992$). Comparison between normal group D treated with A. n. subalata extract and normal control group A showed no significant difference in fasting blood glucose levels (4.52 ± 0.188 vs 4.53 ± 0.185 mmol/l, $p = 1$).

Ethical consideration

This study was performed in accordance with the guidelines for care and use of laboratory animals as prepared 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) [20].

Table 1: effect of Acacia nilotica subalata on fasting blood glucose

Groups 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 ± 2.17 **bc**	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.

Changes in the body mass of diabetic and normal rats

The weight changes between control and experimental groups are shown in table 6. At day 0 and 14 in all groups compared to the normal control group A there was no statistically significant difference between body weights ($p > 0.05$). At day 28 and 42 there was statistical difference in reduction of weight between diabetic groups B, C and E compared to normal control group A ($p<0.05$). There was no statistical difference between C and E compared to diabetic group B (263.76 ± 12.71 and 258.58 ± 6.41 vs 273.48 ± 6.37 g, $p > 0.05$). However, when the comparison was done between normal group D treated with A. n. subalata extract and normal control group A there was significant difference in weight (324.06 ± 9.58 vs 372.50 ± 13.32 g, $p < 0.05$). The control group A increased weight, while the normal group D decreased it. Weight loss in terms of percentage: diabetic control group B lost 6.6 % of their initial body mass. Diabetic group C (treated with A. n. subalata extract) and E (treated with metformin) respectively lost 9. 54 % and 11.29%. Normal group D treated with A. n. subalata lost 0.30% of its initial body mass.

Table 6: Effects of A. n. subalata extract on body mass of normal and diabetic rats

		Grams /weeks			
		0	2	4	6
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
weight	Group A	325.92 ± 13.62	346.25 ± 12.37	360.02 ± 12.97	372.50 ± 13.32
	D	328.64 ± 7.22 #**a**	346.20 ± 7.53 #**a**	334.84 ± 9.75 #**a**	324.06±9.58 **ad**
	B	293.02 ±10.22#**a**	289.42± 17.70#**a**	280.88 ± 7.29 **ab**	273.48 ± 6.37 **ab**
	C	291.60±17.75 #**a**	282.92 ±11.21#**a**	269.70 ± 12.39 **ac**	263.76 ±12.71**ac** (#**b**)
	E	291.50 ± 7.04 #**a**	280.10 ±7.18 #**a**	267.24 ± 6.74 **ae**	258.58 ± 6.41 **ae**(#**b**)

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, #**a:** $p > 0.05$ as compared to group A. #**b:** $p > 0.05$ as compared to group B. **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. **ad** $p< 0.05$ group D compared to group A.

DISCUSSION

Alloxan monohydrate commonly used to induce type 2 diabetes mellitus induces selective partial destruction of β cells of the pancreas [4]. This reduction of β cells decreases insulin output with result hyperglycemia leading to type 2 diabetes mellitus.

Fasting blood glucose

In this study Acacia nilotica subalata leaf extract had hypoglycemic effect in diabetic rats. The results are comparable with those of Maqsood et al. (2008) who reported Acacia nilotica nilotica extract to decrease blood glucose in diabetic rabbits. The glucose-lowering effect of Acacia nilotica subalata extract possibly occurs by stimulating the β cells of the pancreas and/or due to its insulin-like activity. This suggests that Acacia nilotica subalata leaf extract at the dose of 800 mg/kg body weight, may induce β cell reconstruction. The exact mechanism for this action remains unclear. The hypoglycemic effect of Acacia nilotica subalata could reside in the polyphenols found in the plant extract [14]. Polyphenols are known to have antioxidant activity [16], a property that could halt damage of the remaining β cells by clearance of circulating reactive oxygen species generated by alloxan. In addition the polyphenols may help regenerate activity of β cells. It has further been reported that polyphenols and tannins have hypoglycemic effect by their inhibitory interactions with α-amylase and α-glucosidase in the gut [15] hence reducing the amount of absorbable glucose in the small intestine.

The euglycemia found in normal group D compared to normal control A may be due to the normal homeostasis of glucose in normal rats which acts through negative feedback systems to maintain blood glucose within the normal range of 70 to 110 mg of glucose per deciliter of blood [9]. The reduction of blood glucose in group E that received metformin results from the mechanism of action of metformin which acts by suppressing glucose production by the liver [23]. In addition to suppressing gluconeogenesis, metformin increases sensitivity to insulin, enhances peripheral glucose uptake and reduces glucose absorption from gastrointestinal tract [5], all actions that lower plasma glucose.

Changes of rat body mass

In our study, alloxan-induced diabetic rats showed a significant loss of weight compared to normal control. This observation is similar to that done by American Animal Hospital Association (AAHA) on diabetic dogs and cats [1]. When the normal rats and diabetic rats were treated with either Acacia nilotica subalata extract or metformin, the reduction of weight was noticeable compared to normal and diabetic control rats. The exact mechanism of action of Acacia nilotica subalata on weight loss is not known, but the effect of Acacia nilotica subalata extract on weight loss seems to be linked to polyphenol fraction found in the plant extract [14]. It has been reported that polyphenols reduce glucose absorption through the small intestine by inhibiting the action of α-amylase and α –glucosidase [15]. It has also been reported also that the polyphenols have shown an inhibitory effect on adipose tissue formation in wistar rats [11]. In this context the weight loss may be the results of those combined effects. Several mechanisms of metformin for weight loss effects have been evaluated. These include reduction in gastrointestinal absorption of carbohydrates, and insulin resistance, induction of an anorectic and lipolytic effect, and decreased level of leptin [8], a hormone integral to body weight regulation. Both A. n. subalata extract and metformin are helpful in weight loss in T2DM.

CONCLUSION

The present study shows that ethanolic leaf extract of A. n. subalata produced antihyperglycemic effect in alloxan-induced diabetic rats, at the dose of 800mg/kg. It reduced significantly body weight of normal and type 2 diabetic rats. Consequently Acacia nilotica subalata leaf extract is a herbal product with good promise and has the potential to combat obesity and lower blood glucose in normal and type 2 diabetes mellitus. Further studies are required to investigate the detailed mechanism of action of Acacia nilotica subalata leaf extract on body weight and its effects on internal organs.

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