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EFFECTS OF CRUDE EXTRACTS OF *ABELMOSCHUS ESCULENTUS* ON ALBUMIN AND TOTAL BILIRUBIN OF DIABETIC ALBINO RATS

Uraku, A. J[1]., Ajah, P. M[1]., Okaka, A. N. C[2], Ibiam, U. A[1] and *Onu, P. N[3]
[1] Department of Biochemistry/Biotechnology, Ebonyi State University.
[2] Department of Biochemisrty Nnamdi Azikiwe University Awka, Anambra State, Nigeria.
[3] Department of Animal Science, Ebonyi State University, PMB 053 Abakaliki, Ebonyi State Nigeria.
*Corresponding author email: nnenwamazi@yahoo.com

ABSTRACT
The effects of crude extracts of *A. esculentus* on albumin and total bilirubin levels of diabetic albino rats were investigated. Sixty (60) albino rats were divided into four (4) groups of namely A, B, C and D. While A and B groups served as positive and negative controls, respectively, groups C and D served as the experimental groups and each was further subdivided into three (3) groups of 5 animals each. Hyperglycemia was induced in the treatment sub-groups by a single intraperitoneal injection of alloxan at 75 mg per kg body weight. The extracts were administered via oral intubation to the subgroups of C and D in and doses of 200,400 and 800 mg/kg body weight respectively for a period of 14 days. Blood samples were collected through tail vein on various stages and serum glucose, albumin and total bilirubin concentrations were assayed using spectrophotometric method. Results showed that administration of *A. esculentus extract* to diabetic rats group recorded significantly higher ($p<0.05$) body weight gain than those of the test-control and control. There was a significant ($p<0.05$) increase in serum glucose of the untreated diabetic group compare to the control group. Administration of aqueous-ethanolic extracts caused a marked ($p<0.05$) increased in the albumin levels in the diabetic rats. There was a significant ($P<0.05$) increase (82%) in total bilirubin levels in diabetic control group over the normal control. Administration of aqueous-ethanolic extracts caused a marked significant ($p<0.05$) decrease in the billrubin levels in the diabetic rats.

KEY WORDS: *Abelmoschus esculentus*, Diabetic mellitus, hypoglycemic effect, Serum albumin and total bilirubin.

INTRODUCTION

Plant has been the source of help, survival and good health. Plants are able to be functioning in this capacity because they have many important chemical substances found all over their various parts such as alkaloids, carbon compounds, nitrogen, glycosides, essential oils, fatty oils, resins, mucilage, tannins, gums and others (Osmund, 2001). Most of these are potent bioactive compounds found in medicinal plant parts that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (Sofowora, 1993).

The active principles differ from Plants to plants due to their biodiversity and they produce a definite physiological action on the human body. Also, there is growing interest on the medicinal properties of a number of common plants (Gbile and Adosina, 2003). Lawal *et al.* (2006) have elucidated the importance of these medicinal plants and their importance in the pharmaceutical industries. These plants have been under utilized in orthodox medicine but have continued to be used in ethno-medicinal preparations. Today about 300 species of medicinal and aromatic plants are used worldwide in the pharmaceuticals, foods, cosmetics and perfume industries (Osmund, 2001; kafaru, 1984). *Abelmoschus. esculentus*, a flowering plant in the mallow family mavaceae, originating somewhere near present day Ethiopia (Abyssina) (Keay, 1989) but spread to the other continents via African slave trade route (Keay, 1989: Schipper,2000). It was formally considered a species of Hibiscus but now classified in the genus of *Abelmoschus*. It was grown throughout the tropical and warm temperate regions of the world (Schipper, 2000). Due to the nutrient content of the *A. esculentus*, the pods, flowers, leaves and fruits are used as therapeutic diets. In countries such as Turkey and Cyrus, the plants are used in preparing medicinal remedies to and reduce swelling; inflammation (Olusola et al., 2006). The consumption has been reported to reduce serum cholesterol, triacylglyceride and blood pressure (Reid, 1990: Andullu and Varderchyulu, 2001). In some part of Africa such as Ethiopia, the parts are used as part of therapeutic diet against menstrual pains and for hypertension (Daly, 1997). In the preset study we investigated the effects of the ethanoic and aqueous extracts of *A. esculentus* fruits on serum glucose, albumin and total bilirubin in albino rats.

MATERIALS AND METHODS

Collection and Preparation of A.esculentus Extracts

Young fruits of *A. esculentus* were collected from Ogboji - Agoutu in Inyaba Development Centre of Ebonyi State, Nigeria and identified by a plant taxonomist in the Department of Zoology, Ebonyi State University,

Abakaliki. They were washed thoroughly under running tap water, shade dried and pulverized, using a grinding machine. Aqueous and ethanolic extracts were prepared by soaking about 100g of the powdered leaves in 1500ml and 800ml of water and ethanol, respectively for 24hours. The extract was filtered and the solvents removed with rotary evaporations to obtain crude active ingredient.

Experimental Animal

Sixty (60) albino rats of both sexes each weighing 122.50 – 165.50g were procured from The Pharmacy Department of University of Nigeria, Nsukka, Nigeria. The animals were acclimatized for 7 day under standard environment conditions and fed *ad-libitum* on their normal diets. All animals were fasted before the start of the experiment. The animals were distributed into four (4) groups (A, B, C and D). Groups C and D were subdivided into three (3) groups with each sub-group having four animals. Group A was Normal control, Group B, Diabetic control, Group C: Diabetic rats treated with aqueous extract of *A. esculentus* and Group D: Diabetic rats treated with ethanolic extract of *A. esculentus*.

Each animal for diabetic assay was induced by a single intraperitoneal injection of alloxan solution at a dose of 75mg/kg body weight after an overnight fast to groups B, C and D. However, the animals in group A did not receive alloxan dosage and served as animal control. The blood glucose level of the animals was checked using a glucometer (a one touch test strips) after alloxan injection using a method described by Tietz (2000). The blood glucose level of the animals were again checked after 7 days to ascertain a diabetic state, and rats with moderate diabetes were used for the experiment.

Experimental design: In this experiment, a total of 56 albino rats (20 diabetic surviving rats, 10 normal rats) were used. Diabetes was induced in rats a week before the start of the experiment. The rats were divided into four groups after the induction of diabetes. Varying concentrations of the crude extracts of *A. esculentus* were administered via oral intubation to the animals in groups C and D subgroups (C_1, C_2, C_3 and D_1, D_2, and D_3) for a period of 14 days. These served as diabetic experimental groups while those in group B did not receive the extracts and served as diabetic control.

Group A : Normal untreated rats.

Group B : Diabetic untreated rats

Group C_1 : Diabetic rats given aqueous extract of *A. esculentus* (200mg/kg body weight) daily using a canular for 14 days

Group C_2 : Diabetic rats given aqueous extract of *A. esculentus* (400mg/kg body weight) daily using a canular for 14 days.

Group C_3 : Diabetic rats given aqueous extract of *A. esculentus* (800mg/kg body weight) daily using a canular for 14 day

Group D_1 : Diabetic rats given ethanolic extract of *A. esculentus* (200mg/kg body weight) daily using a canular for 14 days

Group D_2 : Diabetic rats given ethanolic extract of *A. esculentus* (400mg/kg body weight) daily using a canular for 14 days.

Group D_3: Diabetic rats given ethanolic extract *of A. esculentus* (800mg/kg body weight) daily using a canular for 14 day Blood samples were collection from the rats at various stages of the experiment namely, at the initial stage after acclimatization, 72hours after injection of alloxan and 14 days after administration of the extracts. Blood samples were collected from the animal via the tail vein under mild anesthesia with chloroform.

Determination of Serum Glucose, total Bilirubin and Albumin

Serum glucose levels were determined in rats using a method described by Tietz (2000).Total serum bilirubin was determined using a modification Tietz (2000) and Jendrassik and Grof (1938). Spectroscopic determination of serum albumin levels was carried out based on a modification of Tietz (2000) and Grant *et al*., (1987).

Statistical Analysis

All the results obtained were expressed as mean ± S.D of 5 rats in each group.All the tested parameters were subjected to statistical analysis using ANOVA. Differences between means were regarded significant at P<0.O5

RESULTS

The effect of the aqueous and ethanolic extracts of *A. esculentus* on the Weight changes and serum glucose levels of albino rats is shown in Table 1. There was a significant ($p<0.05$) decrease in weight gain of the untreated diabetic group compare to the control group. Administration of *A. esculentus extract* to diabetic rats group recorded significantly higher ($p<0.05$) body weight gain than those of the test-control and control. There was a significant ($p<0.05$) increase in serum glucose of the untreated diabetic group compare to the control group.

Administration of aqueous-ethanolic extracts *Abelmoschus esculentus* at 200, 400 and 800 mg/kg to the alloxan induced diabetic rats caused a significant ($p<0.05$) decrease in fasting serum glucose levels of the rats. Administration of aqueous extract caused a marginal ($p<0.05$) reduction in serum glucose of the treated diabetic group compare to the ethanolic extracts treated group Effect of the aqueous and ethanolic extracts of *A. esculentus* on the serum albumin and total bilirubin levels of albino rats is shown in Table 2. The treated animals had significantly lower ($p<0.05$) plasma albumin levels, compared to the normal-control. Albumin level also decreased significantly ($p<0.05$) by 43% in diabetic control group compared to normal control group. Administration of aqueous-ethanolic extracts caused a marked ($p<0.05$) increased in the albumin levels in the diabetic rats. There was a significant ($P<0.05$) increase (82%) in total bilirubin levels in diabetic control group over the normal control. Administration of aqueous-ethanolic extracts caused a marked significant ($p<0.05$) decrease in the billrubin levels in the diabetic rats.

Table 1: Weight changes and serum glucose levels of albino rats after administration of *A. esculentus* extracts

Groups	Initial weight (g)	Final weight (g)	Initial glucose(mg/dl)	Final glucose(mg/dl)
A	165.50 ± 26.73	168.0 0 ±26.50	89.50 ± 2.50	94.00 ± 3.67
B	122.50 ± 4.94	93.75 ± 4.15*	223.75 ± 16.35	241.25 ± 17.46
C_1	165.50 ± 16.45	193.75 ± 14.31#	231.25 ± 7.40	208.75 ± 9.60#
C_2	155.00 ± 18.37	185.75 ± 15.63#	232.50 ± 12.50	197.50 ± 11.46#
C_3	148.75 ± 16.45	187.50 ± 4.82#	233.70 ± 9.60	195.00 ± 12.75#
D_1	139.50 ± 10.31	167.25 ± 13.92#	232.50 ± 5.59	217.50 ± 10.54#
D_2	154.25 ± 8.61	178.75 ± 2.17#	228.75 ± 5.45	202.50 ± 5.59#
D_3	160.75 ± 14.96	188.25 ± 13.20#	235.00 ± 9.35	200.00 ± 13.69#

Values are mean ± SD, n = 5 animals per group. *: Significantly different from the control group ($p<0.05$). #: Significantly different from the diabetic group ($p<0.05$).

Table2: Serum albumin and total bilirubin levels of albino rats after administration of *A. esculentus*.

	Parameters	
Groups	Albumin (g/dl)	Total bilirubin(mg/dl)
A	10.18 ± 0.55	1.98 ± 0.33
B	5.03 ± 0.48*	3.60 ± 0.32*
C_1	6.15 ± 0.48#	2.93 ± 0.29#
C_2	6.33 ± 0.44#	2.88 ± 0.24#
C_3	6.40 ± 0.44#	2.70 ± 0.19#
D_1	5.98 ± 0.47#	3.03 ± 0.29#
D_2	6.10 ± 0.51#	2.90 ± 0.31#
D_3	6.18 ± 0.52#	2.87 ± 0.33#

Values are mean ± SD, n = 5 animals per group. *: Significantly different from the control group ($p<0.05$). #: Significantly different from the diabetic group ($p<0.05$).

DISCUSSION

The effectiveness of ethanolic and aqueous extracts of the leaf of *Abelmoschus esculentus* in reducing blood glucose level in diabetic rats and its effects on blood albumin and bilirubin levels were investigated and compared.The percentage yields of the ethanolic and aqueous extracts were 21.00 and 25.00 respectively. This shows that a substantial fraction of the constituents is freely soluble in ethanol and water. This could explain the use of the aqueous extracts of most medicinal plants by traditional medicine practitioners for the sole purpose of achieving high yield and potency.

The observed significant decrease in the mean body weight in group B may be ascribed to the reduction in utilization of food and fluid. However, Induction of hyperglycemia has been linked to weight loss (Nelson and Cox, 2000). The animals in experimental groups recorded significant ($p< 0.05$) weight gains compared to diabetic control groups (Table 1). Plant extracts effects may be due to a greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence to suggest that plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects. This observation agrees with the report of Agbafor and Akubugwo, 2006which state that the increase in mean body weight of animals given lemon grass is a result of increase in utilization of food and fluid.

A significant ($p< 0.05$) elevation of 150% and 157% in serum glucose was observed in diabetic control when compared with normal control. This increase indicates uncontrolled hyperglycemia in alloxan induced animals. Administration of aqueous-ethanolic extracts *Abelmoschus esculentus* at 200, 400 and 800 mg/kg to the alloxan induced diabetic rats caused a significant ($p<0.05$) decrease in fasting serum glucose levels with percentage values of 13 and 10%; 18 and 16%; 19 and 17%, respectively. This suggests that the extracts contain principles with the potential of reducing glucose level in serum. The marginal reduction in serum glucose achieved with aqueous extract at the various levels indicates that aqueous extract is more effective in reducing srum glucose level than ethanolic extract. This observation is in agreement with the report of Iwueke *et al*. (2006) that aqueous extract was more efficient in reducing blood glucose level than the ethanolic extract.

Albumin level also decreased significantly ($p<0.05$) by 43% in diabetic control group compared to normal control group. This observation may be attributed to inducement of hyperglycemia by alloxan. Hyperglycemia increased gluconeogenesis and thereby resulting to excess protein breakdown and also excess nitrogen loss due to gluconeogenesis (Robert *et al.,* 2000).

However, administration of aqueous-ethanolic extracts caused a remarkable increased in the albumin levels in the diabetic rats with percentage change values of 22 and 19%; 26 and 21%, and 27 and 30 % for 200, 400 and 800 mg/kg weight doses, respectively. These observations may be due to the presence of some compounds which help in provision of a reserved store of protein (Nsirim, 1999; Kaplan *et al.,* 1999). However, the extracts mechanisms

of action behind these observations remain to be determined although the mechanism suggested here may not be ruled out completely.

There was a 82% increase in total bilirubin levels in diabetic control group. On treatment with the extracts, the levels decreased by 19 and 16%; 20 and 26 and, 22 and 20% ($P<0.05$) of that of diabetic control. This indicates that hyperglycemia can enhance protein glycation. The increased total bilirubin levels indicate that excess haemoglobin is being destroyed or that the liver is not actively treating the haemoglobin it is receiving (Andullu and Vardycheryalu, 2001 and Tietz, 2000).

In conclusion, the results of this study suggest that the fruits of *Abelmoschus esculentus* extracts possess hypoglycemic potential. However, aqueous extracts is more effective in controlling gluconeogenesis.

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