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THE EFFECTS OF *ABELMOSCHUS ESCULENTUS* FRUITS ON ALP, AST AND ALT OF DIABETIC ALBINO RATS

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ABSTRACT
The effects of A. *esculentus* fruits on Alkaline phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities on diabetic albino rats were investigated. The animals were grouped into four (4): A, B, C and D. Group C and D were subdivided into three group each. Diabetes mellitus was induced by single intraperitoneal injection of alloxan 75mg/kg body weight. The extracts were administered via oral intubation to C and D groups and varying concentrations of 200, 400 and 800mg/kg body weight of the extracts respectively was given to each subgroup for a period of 2 weeks. Physical activities, food and fluid intake, and body weight increased after administration of the extracts. These parameters; serum glucose levels, and activities of enzymes viz ALP, AST and ALT decreased significantly ($P<0.05$) after administration of the extracts. These elevations and reductions were found to be dose dependent. The results support the therapeutic use of *A. esculentus as* antidiabetic plant and are non – toxic to Liver enzymes.

KEY WORDS: *Hibiscus esculentus*, Herbaceous plant, Therapeutic diet, Hyperglycemic effect, hypoglycemic effect, Liver enzymes.

INTRODUCTION

One of the major uses of plant apart from food, clothing, shelter and timber is its therapeutic uses (Atawodi *et al.*, 2003). Much of the medicinal uses of plants seem to have developed through observations of their effects on domestic animals, which were most often by trial and error (Olajide *et al*., 2000). The active ingredients of most of the commonly used conventional drugs were originally derived from plant parts before their pharmaceutical mass production from synthetic chemical (Holetz, 2002; Odeku *et al*., 2008). Presently, the use of natural products ranging from drugs and foods for industrial material is increasing (Okeke and Elekwa, 2006). This is because of the fear of side effects, environmental pollution and other unfavorable factors that are often associated with conventional medications which are relatively reduced or even absent in natural products (Batista *et al*., 2009). In addition, some ailments do not have any available conventional or preventive drugs. This therefore makes research in plants and other natural sources very imperative. Many locally available plants acclaimed to possess some therapeutic properties are being subjected to scientific study to ascertain the basis of their therapeutic effects and possibly be integrated into modern medicinal practice (Uraku *et al*., 2009)

Abelmoschus esculentus, a member of the family Malvaceae is an annual or perennial herbaceous plant growing up to 2m tall. It is grown throughout the tropical and warm temperate regions of the world for its fibrous pods full of seeds, which when picked young are eaten as a vegetable Schipper, 2000). The plant which is of African origin was brought to United State via African slave trade, and is grown in the Southern State as an annual crop (Uraku *et al.*, 2010). 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 Cyprus, the plants are used in preparing medicinal remedies to and reduce swelling; inflammation ((Ijeh *et al*., 2005)

The consumption has been reported to reduce serum cholesterol, triacylglyceride and blood pressure (Andullu and Vardacharyulu, (2001). In some part of Africa such as Ethiopia, the parts are used as part of therapeutic diet against menstrual pains and for hypertension (Salawu, 2009). This paper therefore dealt with a comparative study on the effect of the ethanolic-aqueous extracts of A. esculentus fruits on serum blood glucose, ALP and Aminotransferases.

MATERIALS AND METHODS

Collection and Preparation of *A. esculentus* Extracts

The study was carried out on the Month of November, 2009. 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 Effects of *Abelmoschus esculentus* on ALP, AST and ALT activities on diabetic rats 2 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 was procured from The Pharmacy Department of University of Nigeria, Nsukka, Nigeria. The animals were acclimatized for 7 day under standard environmental 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 75 mg/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* (200 mg/kg body weight) daily using a canular for 14 days

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

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

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

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

Group D_3: Diabetic rats given ethanolic extract *of A. esculentus* (800 mg/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, 72 hours 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, Alkaline phosphatase (ALP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities:

Serum glucose levels were determined in rats using a method described by Tietz (2000). Alkaline phosphatase (ALP) was determined using a described by Deutsche, 1972 and Aminotransferases (AST, ALT) activities were determined in rats using a method described by Reitman and Frankel, (1957)..

Statistical analysis

All the tested parameters were subjected to statistical analysis using T-test. Differences between means were regarded significant at P<0.O5.

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 (151)	89.50 ± 2.50	94.00 ± 3.67
B	122.50 ± 4.94	93.75 ± 4.15* (23.47)	223.75 ± 16.35 (150)	241.25 ± 17.46 (157)
C_1	165.50 ± 16.45	193.75 ± 14.31# (19.07)	231.25 ± 7.40	208.75 ± 9.60# (13)
C_2	155.00 ± 18.37	185.75 ± 15.63# (19.84)	232.50 ± 12.50	197.50 ± 11.46# (18)
C_3	148.75 ± 16.45	187.50 ± 4.82# (26.05)	233.70 ± 9.60	195.00 ± 12.75# (19)
D_1	139.50 ± 10.31	167.25 ± 13.92# (19.89)	232.50 ± 5.59	217.50 ± 10.54# (10)
D_2	154.25 ± 8.61	178.75 ± 2.17# (15.88)	228.75 ± 5.45	202.50 ± 5.59# (16)
D_3	160.75 ± 14.96	188.25 ± 13.20# (17.11)	235.00 ± 9.35	200.00 ± 13.69# (200)

Values are mean ± SD, n = 5 animals per group. *: Significantly different from the control group, (A) at p<0.05. #: Significantly different from the diabetic group (B) at p<0.05. Figures in parenthesis indicate percent increase/decrease

TABLE 2. Enzyme activities of Albino Rats after administration of *A. esculentus* extracts

Groups	ALP (µ/L)	AST (µ/L)	ALT (µ/L)
A	165.00±73.79	14.50±3.35	15.50±3.77
B	521.50±76.45* (216)	80.75±9.20* (457)	80.75±9.20* (469)
C_1	412.50±120.12# (21)	58.50±10.97# (28)	71.00±7.78# (20)
C_2	396.00±33.00# (24)	51.50±7.97 # (36)	68.25±5.45# (23)
C_3	379.50±28.58# (27)	48.50±8.38# (40)	60.75±4.15# (31)
D_1	453.75±85.34 # (13)	63.50 ±8.96# (21)	74.75±5.93# (15)
D_2	437.25±94.43# (16)	58.50±10.97# (28)	70.75±6.50# (20)
D_3	429.00±16.67 # (18)	56.25±7.53# (30)	63.25±4.15# (28)

Values are mean ± SD, n = 5 animals per group. *: Significantly different from the control group, (A) at p<0.05. #: Significantly different from the diabetic group (B) at p<0.05. Figures in parenthesis indicate percent increase/decrease

RESULT AND DISCUSSION

Ethanolic extraction of the homogenized dried samples gave a relatively low percentage yield than the aqueous extract. This suggests that most of the chemical components of the plant have low solubility in ethanol.

The effect of the aqueous and ethanolic extracts of *A. esculentus friuts* on the Weight changes and serum glucose levels of albino rats are shown in Table 1. There was a significant ($p<0.05$) decrease in weight gain from 122.50 ± 4.94 - 93.75 ± 4.15 which represent 23.47% change in the untreated diabetic group when compare to the control group. This observation may be attributed to the reduction in utilization of food and fluid. However, inducement of hyperglycemia has been linked to weight loss (Ugochukwu and Babady, 2003). Administration of different doses viz; 200, 400 and 800 mg/kg of *A. esculentus aqueous – ethanolic extract* to diabetic rats group recorded significantly higher ($p<0.05$) body weight gain as follows 165.50 ± 16.45 - 193.75±14.31, 155.00 ± 18.37 - 185.75±15.63, 148.75 ± 16.45 - 187.50 ± 4.82, while as ethanolic extract shown 139.50 ± 10.31 - 167.25 ± 13.92, 154.25 ± 8.61- 178.75 ± 2.17, 160.75 ± 14.96 - 188.25 ± 13.20 with % change of 19.07, 19.84, 26.05, 19.89, 15.88 and 17.11 respectively than those of the test - control and control. The results agree with the report of Agbafor and Akubugwo, 2006 which states that the increase in the mean body weight of the animals given Lemon grass is as a result of increase in utilization of food and fluid. Also, the observation is line with the report of Okeke and Elekwa, 2006

There was a significant ($p<0.05$) increase in serum glucose; 223.75 ± 16.35 - 241.25 ± 17.46 which represent a significant elevation 150% and 157 of the untreated diabetic group compare to the control group. This increase indicates uncontrolled hyperglycemia in the alloxan induced animals (table 1).

Mean serum glucose concentrations in aqueous - ethanolic extracts of the treated groups were significantly ($P<0.05$) decreased by 10 and 13%; 16 and 18%; 17 and 19% when compared to diabetic control when the plant extracts were administered at different doses; 200, 400 and 800mg/kg body weight respectively. This indicates that aqueous extract of the plant is more effective in controlling hyperglycemia than the ethanolic extract. The hypoglycemic activities of the extracts could be attributed to the presence of some compound which phytochemical studies have revealed to contain by the plant extracts (Iwueke *et al.*, 2006; Ara, *et al.*, 2008). The observation is line with the report of Andullu and Vardacharyulu, (2001).

There was a significant ($P<0.05$) increase by 216%, 457%, 469% in ALP, AST and ALT activities in diabetic control group over the normal control (Table 2). This observation agrees with the report of Andullu and Vardacharyulu, 2001; Iweala and Obidoa, 2009 which states that during diabetes intestinal ALP leached from intestines and translocated to the circulation.

A esculentus fruits treatment brought down such elevated levels of ALP significantly ($P<0.05$) by 13 and 21 %, 16 and 24%, and 18 and 27% in diabetic animals on administration of varying dosages; 200,400 and 800mg/kg body weight of the aqueous – ethanolic extracts respectively. This result could be due to certain compound present in the plant extracts which undergo exchange reactions with titrable - SH groups of the enzyme and proteins in the body spontaneously and inhibit the enzyme activity (Ahmed and Sharma, 1997). This agrees with a study on the effect of 3- allyl sulphoxide isolated from garlic alloxan diabetic rats (Sheela and Augusti, 1992; Nwanjo and Alumanah, 2005). Accelerated gluconeogenesis, negative nitrogen balance and muscle wasting are among the hallmarks of uncontrolled hyperglycemia (Busa *et al.*, 1972; (Ugochukwu and Babady, 2002). There is a catabolism of branches amino acids and alanine release by skeletal muscle (Odessey *et al.*, 1997; George and Chaturvedi, 2007). Glutamate is an obligate precursor of alanine and glutamine production by muscles. The latter two amino acids comprise more than 50% of all the amino acids released by the muscle, alanine being the preferred amino acid of precursor of gluconeogenesis in liver and glutamine in the kidney (Cahill *et al.*, 1972; Tietz, 2000) It is evident from table 2 that the activity of AST and ALT were enormously elevated ($P<0.05$) by 457% and 469% respectively in diabetes (groups B) from that of normal group A, indicative of enhanced gluconeogenesis in uncontrolled diabetes. *A esculentus* fruits trea tment significantly ($P<0.05$) decreased the activities of AST by 21 and 28%, 28 and 36% and 30 and 40% while 15 and 20%, 20 and 23% and 28 and 31% decrease were only noticed in ALT. These changes were observed when the varying concentrations of 200,400 and 800mg/kg body weight of the extracts were administered to the animals respectively. These observations are in accordance with a study on the effect of mulberry leaves on diabetes (Adedapo *et al.*, 2009). These reductions in serum enzymes activities were dose dependent. These suggest that the plant extracts can control the rate of gluconeogenesis in diabetes but is more effective in aqueous extracts.

CONCLUSSION

The results revealed that the plant, *Abelmoschus esculentus* has hypoglycemic effect and can be used in management/treatment diabetes. Thus, the plant no toxic effect on the Liver enzymes via Serum ALP and aminotransferases.

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