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Possible Evidence of Gluconeogenesis in Rabbits Given Cashew (*Anacardium occidentale*) Leaf Extract

Mathew Folaranmi OLANIYAN*, Temitayo AFOLABI
Department of Medical Laboratory Science, Achievers University, Owo – Nigeria

Original Research Article

***Corresponding author**
Mathew Folaranmi OLANIYAN

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Abstract: Gluconeogenesis involves formation of glucose from the breakdown of protein to form glycogenic amino acid and lipid such as triglyceride to generate glycerol which is further utilized for the formation of glucose. Cashew (*Anacardium occidentale*) leaf has some non-scientific but traditional health benefit claims such as in the treatment of diabetes and hypertension. This work was designed to determine the evidence of Gluconeogenesis in rabbits given ypung cashew leaf extract using Plasma Value of Cortisol, Glycerol, Cortisol Binding Globulin Glucose, Total Triglyceride and Total Bile Acid. Materials and Methods: 10 rabbits of the same sex weighing 1.0-1.2Kg grouped into A1 (5 rabbits fed with normal meal and water only for 7 days), A2 (A1 rabbits given 500mg/KgBW of ethanolic extract for another seven days) and B1 (5 rabbits fed with normal meal and water only for 7 days), B2 (A1 rabbits given 500mg/KgBW of aqueous extract for another seven days). Plasma cortisol, glycerol, cortisol binding globulin, glucose, total triglyceride and total bile acid were measured biochemically using auto-analysis, ELISA and colorimetric techniques. The result obtained showed a significantly higher plasma value of cortisol, Glycerol and a significantly lower mean plasma value of Glucose and Total Triglyceride in rabbits (A2 and B2) given 500mg/KgBW of ethanolic or aqueous extract for seven days compared with the values of these parameters obtained in the same rabbits when they were fed with normal meal and water only for 7 days (A1 and B1) with $p<0.05$. This work reviled possible evidence of gluconeogenesis as indicated by the significant biochemical alterations the plasma values of cortisol, glycerol, glucose and total triglyceride with respect to changes in their plasma level before and after the supplementation of the extract of young cashew (*Anacardium occidentale*) leaf.

Keywords: Gluconeogenesis, Rabbits, *Anacardium occidentale*, Leaf Extract, Plasma, Cortisol, Glycerol, Cortisol Binding Globulin, Glucose, Total Triglyceride, Total Bile Acid.

INTRODUCTION

Gluconeogenesis is the synthesis of glucose from non-carbohydrate source such as from the breakdown of protein which include Glycogenic amino acids (Amino Acids that can be converted into glucose) and breakdown of lipids such as triglyceride. The Lipolysis of triglyceride will form fatty acid and glycerol [1-8]

The glycerol is phosphorylated under the influence of Glycerol Kinase to form Glycero-3-phosphate which can further be metabolized to form glucose [1-8]. Gluconeogenesis is stimulated by decreased plasma glucose level. Decrease in Plasma Glucose level stimulates the release of a glucocorticoid steroid hormone known as cortisol from zona fasciculata of the adrenal cortex in the adrenal gland [1-8].

Cortisol is an anti-stress hormone which binds and store in a protein known as Cortisol Binding Globulin in the body. Gluconiogenesis takes place in the liver. Total Bile acid is an index of Hepatobiliary diseases [9-13].

Young cashew (*Anacardium occidentale*) leaf raw liquid extract is applied traditionally in the treatment of hypertension in south western part of Nigeria. It contains Anacardic acid, flavonoid, tannins, flavonoids, phenolic compounds, cardols, triterpenoids, methylcardols, xantoprotein and cardanols. It has a traditional claim as an anti-microbial agent, treatment of diabetes mellitus and increase in sugar absorption and utilization by cells [14]. Young cashew leaf extract has been demonstrated to decrease high blood pressure and has a cholesterol/lipids lowering effect [15].

This work was designed to determine the evidence of Gluconeogenesis in rabbits given ypung

cashew leaf extract using Plasma Value of Cortisol, Glycerol, Cortisol Binding Globulin Glucose, Total Triglyceride and Total Bile Acid.

MATERIALS AND METHODS

Study area

Animal house of Achievers University, Owo-Nigeria equidistant between Nigeria Federal capital territory-Abuja and former Federal capital-Lagos. It has a Latitude: 6.98575 , Longitude: 5.27103 and Time Zone: UTC+1, Africa/Lagos.

Study population

Rabbits were bought from Oja Ikoko-a major market in Owo and were identified and confirmed having same sex (male) in the Department of Biological Sciences, Achievers University, Owo-Nigeria. This include 15 rabbits with weight ranging from 1.0-1.2Kg grouped as follows:

Group A_1: Five rabbits weighing 1.1 ±0.1 Kg fed with normal meal and water were studied as control group A_1.
Group A_2: Five rabbits (A_1) Given 500mg/KgBW of ethanolic extract for another seven days.
Group B_1: Five rabbits weighing 1.1 ±0.1 Kg fed with normal meal and water were studied as control group A.
Group B_2: Five rabbits (B_1) Given 500mg/KgBW of aqueous extract for another seven days

Preparation of the Cashew Extracts

Cashew leaves were plucked from major farms in and around Owo-Nigeria and was identified by the Department of Biological Sciences. The leaves of Cashew leaves were air dried for 14 days, Ethanolic and aqueous extraction was carried out by soaking 50g of powers of Cashew leaves into 500ml of each of ethanol and sterile distilled water for 24hours. Following the report of Das *et al.* [16] that solvent to sample ratio of 10:1 (v/w; solvent to dry weight ratio) has been used as ideal. Each extract was filtered through Whatmann filter paper No.1 and filtrates concentrated at room temperature in order to reduce the volume. Further concentration and drying by volume extraction was carried out using rotary evaporator and stored in refrigerator prior to use. Four hundred milligramme of the extract powder was dissolved in 2ml of distilled water for administration.

Blood specimen

Fasting Blood samples were collected from the veins lining the ear of the rabbits after each treatment into lithium heparinized bottles for the estimation of Total cholesterol, LDL cholesterol and Total triglycerides.

Determination of Biochemical Parameters

Plasma concentration of Total Triglycerides, Glucose, Glycerol, Cortisol, Cortisol Binding Globulin, Total Bile acid

Method of Data analysis

The results obtained was subjected to statistical analysis using SPSS 18.0

Ethical Consideration

The rabbits were treated and sacrificed in line with the ethical guideline as provided by Research and Ethical Committee of the Department of Biological Sciences, Achievers University, Owo-Nigeria.

Method of analysis of biochemical parameters

Plasma Glucose and Total triglyceride

Plasma Glucose and total triglyceride were analysed in the rabbits using chemistry auto-analyzer Colbas C111 using ROCHE reagent.

Corticosteroid Binding Globulin (CBG)

This was carried out using Corticosteroid Binding Globulin (CBG) MYBIOSOURCE ELISA Kit. MBS2600190 is a ready to use microwell, strip plate ELISA (enzyme linked immunosorbent assay) Kit for analyzing the presence of the Corticosteroid Binding Globulin (CBG) ELISA Kit target analytes in biological samples. The concentration gradients of the kit standards or positive controls render a theoretical kit detection range in biological research samples containing CBG. The ELISA analytical biochemical technique of the MBS2600190 kit is based on CBG antibody CBG antigen interactions (immunosorbency) and an HRP colorimetric detection system to detect CBG antigen targets in samples. The ELISA Kit is designed to detect native, not recombinant, CBG. Appropriate sample types may include undiluted human body fluids and/or tissue homogenates, secretions. Quality control assays assessing reproducibility identified the intraassay CV (%) and interassay CV(%).

Plasma Cortisol

This was carried out by ELISA technique using Rabbit Cortisol ELISA Kit of Hcusabio.

Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Cortisol. Standards or samples are added to the appropriate microtiter plate wells with Biotin-conjugated Cortisol. A competitive inhibition reaction is launched between Cortisol (Standards or samples) and Biotin-conjugated Cortisol with the pre-coated antibody specific for Cortisol. The more amount of Cortisol in samples, the less antibody bound by Biotin-conjugated Cortisol. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of Cortisol in the sample. The color development is stopped and the intensity of the color is measured.

Total Bile Acid

Estimation of Total Bile Acids was carried out on the plasma samples of the subjects using Randox reagent kit. The manufacturer's instruction was strictly followed.

Principle

Two reactions are combined in this kineticenzyme cycling method. In the first reaction bile acids areoxidised by 3-α hydroxysteroid dehydrogenase with the subsequent reduction of Thio-NAD to Thio-NADH. In the second reaction the oxidised bile acids are reduced by the same enzyme with the subsequent oxidation of NADH toNAD. The rate of formation of Thio-NADH is determined by measuring the specific absorbance change at 405 nm. (Abreviations: NADH, NAD, Thio-NADH, Thio-NAD).

Measurement of Plasma Glycerol

Measurements are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders. Plasma glycerol was measured in the rabbits using the reagent kit of RANDOX.

Principle

A direct colorimetric procedure for the measurement of glycerol is described utilizing a quinoneimine chromogen system in the presence of glycerol kinase, peroxidase and glycerol phosphate oxidas

RESULTS

The result obtained showed a significantly higher plasma value of cortisol, Glycerol and a significantly lower mean plasma value of Glucose and Total Triglyceride in rabbits (A2 and B2) given 500mg/KgBW of ethanolic or aqueous extract for seven days compared with the values of these parameters obtained in the same rabbits when they were fed with normal meal and water only for 7days (A1 and B1) with $p<0.05$ (Table 1, 2 and figure 1).

There was no significant difference in the plasma value of Cortisol Binding Globulin and Total Bile Acid in rabbits (A2 and B2) given 500mg/KgBW of ethanolic or aqueous extract for seven days compared with the values of these parameters obtained in the same rabbits when they were fed with normal meal and water only for 7days (A1 and B1) with $p>0.05$ (Table 1, 2 and figure 1 and 2).

There was no significant difference in the plasma value of cortisol, Glycerol, Glucose, Total Triglyceride, Cortisol Binding Globulin and Total Bile Acid in rabbits given 500mg/KgBW of ethanolic extract for seven days (A2) compared with rabbits Given 500mg/KgBW of aqueous extract for seven days

Table 1: Mean and Standard Deviation of the Plasma Value of the Biochemical Parameters Obtained in the Rabbits

	Group A1	**Group A2**	**Group B1**	**Group B2**
Cortisol (ng/ml)	80±2.0	122±3.0	81±3.0	125±4.0
Cortisol Binding Globulin (pmol/ml)	158±3.0	160±3.0	161±2.0	160±3.0
Total Bile Acid (µml/L)	2.5±0.2	2.7±0.2	2.6±0.2	2.8±0.2
Glucose (mg/dl)	90±4.0	61±3.0	93±3.0	62±2.0
Glycerol (mcmol/L)	100±5.0	172±4.0	102±6.0	170±3.0
Total Triglycerides(TG-T) (mg/dl)	50.0±1.0	41±2.0	51.0±2.0	40.0±1.0

Table 2: Comparative analysis of the Plasma Value of the Biochemical Parameters Obtained in the Rabbits

		A_1 vsA_2	B_1VsB_2	A_2 Vs, B_2
Cortisol (ng/ml)	"t"	-11.65	-8.88	-0.6
	"p"	0.004**	0.006**	0.30
Cortisol Binding Globulin (pmol/ml)	"t"	-0.47	0.27	0.00
	"p"	0.34	0.40	0.50
Total Bile Acid (µml/L)	"t"	0.71	0.71	-0.35
	"p"	0.28	0.28	0.38
Glucose (mg/dl)	"t"	5.8	8.89	-0.27
	"p"	0.009**	0.006**	0.40
Glycerol (mcmol/L)	"t"	-11.25	-10.13	0.40
	"p"	0.004**	0.005**	0.36
Total Triglycerides(TG-T) (mg/dl)	"t"	4.02	4.92	0.44
	"p"	0.03*	0.02*	0.35

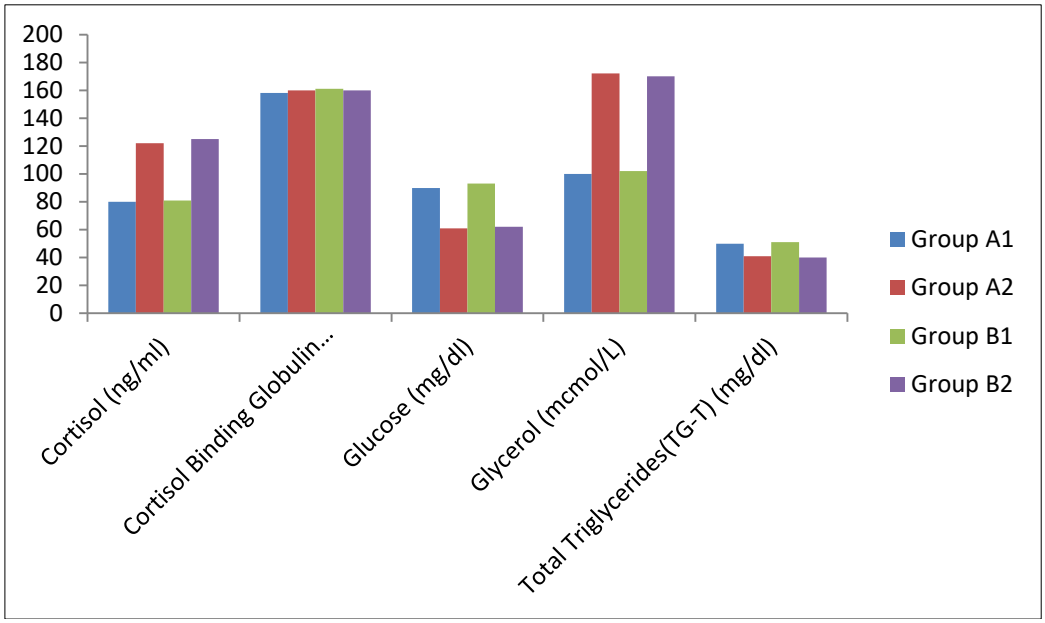

Fig-1: Comparative Description of the Plasma Value of Cortisol, Glycerol, Cortisol Binding Globulin Glucose and Total Triglyceride Obtained in the Rabbits

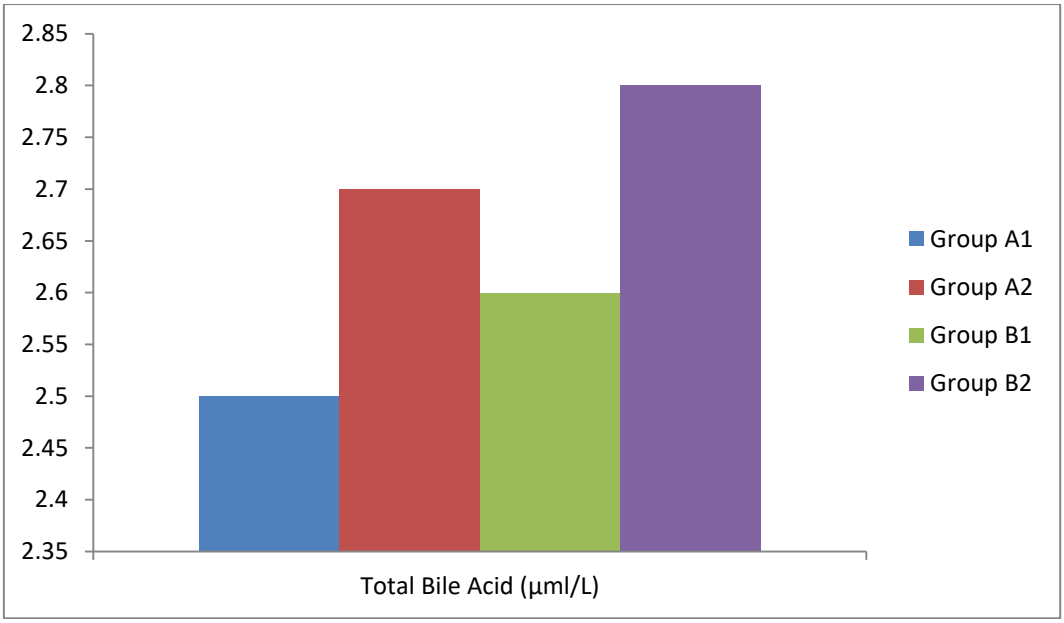

Fig-2: Comparative Description of the Plasma Value of Total Bile Acid Obtained in the Rabbits

DISCUSSION, CONCLUSION AND RECOMMENDATION

The result obtained showed a significantly higher plasma value of cortisol, Glycerol and a significantly lower mean plasma value of Glucose and Total Triglyceride in rabbits (A2 and B2) given 500mg/KgBW of ethanolic or aqueous extract for seven days compared with the values of these parameters obtained in the same rabbits when they were fed with normal meal and water only for 7days (A1 and B1).

Cortisol a glucocorticoid is a steroid hormone and an hyperglycemic agent. Increase in plasma cortisol level following the administration of cashew leaf extract could be associated with increase in the release of cortisol from adrenal cortex as a result of decrease in plasma glucose obtained in the rabbits [9-13]. Cortisol is an anti-stress hormone. [9-13]. It is released when the plasma glucose level is low to stimulate gluconeogenesis involving formation of glucose from the broken down of lipid and protein to form glycerol and glycogenic amino acids that could be converted to glucose to maintain glucose homeostasis [1-8].

This biochemical process (gluconeogenesis) involves excessive utilization of lipid such triglyceride

to generate glycerol which will in turn be phosphorylated under the influence of glycerol kinase, glycerol-3-phosphate generated from this process will now be used biochemically to form glucose. This process could cause an increase in plasma glycerol and a deplete in plasma triglyceride concentrations which could be associated with the findings of this work [1-8].

Furthermore, decrease in plasma glucose following the administration of young cashew leaf in the rabbits is consistent with the report of Jaiswal *et al.*, [17] that the traditional use of *A. occidentale* (Cashew leaf extract) as a hypoglycaemic agent is justified as the extracts from the leaves of this plant show a significant activity which is comparable to the standard hypoglycemic drug pioglitazone.

CONCLUSION

This work reviled possible evidence of gluconeogenesis as indicated by the significant biochemical alterations the plasma values of cortisol, glycerol, glucose and total triglyceride with respect to changes in their plasma level before and after the supplementation of the extract of young cashew **(***Anacardium occidentale***)** leaf.

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