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Assessment of Electrolytes Distribution in Poloxamer-407 (P-407) Induced-Hyperlipidaemic Wistar Rats Treated with Fenugreek Seed SupplementsAbdulrazak, A¹*, Tanko, Y²., Kabiru, A². and Mohammed K.A.¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Kaduna State University, Kaduna Nigeria ¹, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Ahmadu Bello University Zaria, Kaduna Nigeria ².

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Abstract

High fat diet (HFD) contribute to the advent of health problems. Recent studies have indicated that obesity imposes a hemodynamic overload to the kidneys. In Hyperlipidaemia associated with cardiovascular disorders leading to increased morbidity among individuals is alarming. Challenges or of adversed effect of certain antihyperlipidaemic drugs (statins, fibrates, bile acid sequestrant and cholesterol absorption inhibitors), their high cost and unavailable to the population at grass root. There is need to search for an alternative remedy with lesser side effect which is easier accessible to mass population. Twenty (20) Wistar rats were divided in four groups n=5, hyperlipidaemia was induced using a single dose of P407 (1.5g/Kg) IP at the interval of three days. Group 1 served as the normal control, group 2 served as disease control (hyperlipidemic rats placed on normal diet), group 3 comprises of hyperlipidemic rats placed on Cholestyramine (0.26g/Kg) and group 4 comprises of hyperlipidemic rats placed on fenugreek seed supplement (2.5%), after three weeks of treatment blood sample were collected via cardiac puncture from which serum was used for assessment of electrolytes, creatinine and urea using standard procedures. Results indicate a significant decrease in creatinine levels in the supplement treated group compared to the disease control group. This indicates reversal of altered creatinine over excretion and a sign of a better performing renal functions. Fenugreek seed supplements have a protective effect on renal tissue integrated function. With further studies a specific dose may be fine-tuned for human consumption.

Introduction

Plasma electrolytes along with urea and creatinine are important clinical makers of body fluid content and homeostasis. The functional roles interplayed on ingested substances by the liver as the major metabolically active organ and the kidney as the primary excretory organs to maintain the normal concentration of these makers is paramount to the wellbeing of the body. Thus, their physiological profile gives insight on functional status of vital organs including the liver and kidney, while abnormal profile distribution of electrolytes indicates health complications. Certain conditions are associated to abnormal electrolytes profile this include plasma over loading with osmotically driven substrates such as glucose in hyperglycemia and excess lipids in hyperlipidemia that could lead to cardiovascular disorders (Candan *et al.*, 2014).

Electrolytes imbalance contribute to complications observed in diabetes. An important aspect not often discussed in many diabetes studies, is the relationship between the overweight and obesity caused by hypercaloric and high fat diet overload to the development of kidney failure. In fact, a retrospective study revealed a prevalence of 70% of metabolic syndrome among 202 patients with kidney failure admitted to initiate the renal replacement therapy (Innocent *et al.*, 2007).

Hyperlipidaemia refers to elevated levels of lipids and cholesterol in the blood beyond the physiological range (Goce *et al.*, 2005). Alteration and abnormality in the metabolism of

lipid and lipoproteins is on the increase and becoming common condition taken place globally within general population mostly due to abnormal dietary shift. It is considered as one of the main risk factors in the incidence of cardiovascular disease due to their influence on atherosclerosis. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis related conditions like coronary heart disease (CHD), ischaemic cerebrovascular disease, peripheral vascular disease and pancreatitis. The increase in lipids like low density lipoproteins (LDL), cholesterol (esters derivatives) and triglycerides are mainly responsible for this Condition along with distorted electrolytes levels (Dimeski and Barnett, 2005). These lipids are associated with blood plasma proteins and remain in the dissolved state in the blood. The primary reason for hyperlipidemia is defect in lipid metabolism which is caused by the defect in lipoprotein lipase activity or the absence of the surface Apoprotein C-II. Abundance and ingestion of oily-nutrients often leads to hyperglycemia due to hepatic overload of fats.

Poloxamer are nonionic surfactant vehicles. They are a series of chemically-related block copolymers. They have widespread use in parenteral formulations as solubilizing and

wetting agents for traditional, low-molecular weight organic drug molecules, as well as stabilizing agents for proteins and polypeptide drugs (Chunga *et al.*, 2010). Poloxamer 407 has been reported to cause increased and a sustained (greater than 96 hour) plasma cholesterol and triglyceride concentrations in rats when administered intraperitoneally (Zjumira *et al.*, 1992).

The development of medicinal plants in the domain of nutrition has unveiled their additional therapeutic potentials (Ramawat *et al.*, 2009). In the same era, terms like functional foods, nutraceutical and Farm foods have taken hold of the nutrition market, mainly aiming to provide nutrition and healthy diets. Many plants and their components have been extensively investigated for their health-promoting benefits, including antioxidant, cardio-protective, anti-hyperlipidemia, anti-cancer, antimicrobial and immune-modulatory activities (Sharma and Seth, 2004).

Fenugreek is regarded as one of the oldest known medicinal plant in recorded history (Goyal *et al.*, 2016). Its seed and leaves have medicinal value, and have been used to reduce blood glucose and lower blood cholesterol in humans and animals (Dahanukar *et al.* 2000).



Plate 1: Fenugreek (*Trigonella foenum-graceum* C.F) Seeds (Photograph by Author, 2017).

Fenugreek contains three important chemical constituents (steroidal sapogenins, galactomannans and isoleucine) with medicinal value. These constituents have placed fenugreek among the most commonly recognized "nutraceutical" or health food products. Legume consumption is known to have a beneficial or protective effect in diabetes, hypercholesterolemia and coronary heart disease, as well as protecting against obesity and menopause (Iyer *et al.*, 2009).

Various animal models have been used to study the pathophysiological effects of hyperlipidaemia. Genetic variants of hyperlipidaemia, such as the obese Zucker rat are available. Alternatively, hyperlipidaemia can be induced by dietary means (e.g. chronic feeding of a high fat diet) (Jimoh *et al.*, 2015) or by treatment with compounds such as Triton or poloxamer 407 (P407) (Chunga *et al.*, 2010).

Fenugreek seeds have been in used across the globe as natural remedy and in folklore for many disease management. Positive therapeutic effects of many extracts including that of fenugreek have been studied with paucity in results (Alnoory *et al.*, 2013) and little literature on its effects on electrolytes, urea and creatinine levels in hyperlipidemia. Therefore, the present study aimed at determining the effects of fenugreek supplements on electrolytes urea and creatinine levels in P407 – induced hyperlipidemic Wistar rats.

Methodology

Materials

Chemicals/Drugs and other Equipment's; Cholestyrimine (Cholestran powder) (PHARCO Pharmaceuticals, Alexandria, Egypt) purchased from Amira Pharmacy, Tudun Wada Zaria, Kaduna state Nigeria.

Polaxamer 407 (P-407) purchased KEM LIGHT Laboratory PVT. LTD, Mumbai India.

The Fenugreek seeds was purchased from Terminus Jos, Plateau State of Nigeria.

Methylated spirit (500ml), Chloroform (200ml), cotton wool, disposable pipette tips, 1ml and 5ml syringes (20 each), Dissecting Kits, EDTA and Plain bottles (25 each), A pair Scissors, Gloves and Cotton wool were purchased from Steve Moore Chemical Co. LTD, Palladan Zaria,

Kaduna State. Ice block obtained from Department of Human Physiology ABU Zaria, Kaduna state Nigeria.

Methods

Collection and Preparation of Plant

The Fenugreek seeds was purchased from Terminus Jos, Plateau State of Nigeria. The Fenugreek seeds was presented to the herbarium unit of the Department of Botany, Faculty of life Science, Ahmadu Bello University, Zaria, where it was identified by a Taxonomist Malam Namadi Sunusi, with a deposited voucher of 12034.

The Fenugreek seeds was ground into fine powder using laboratory mortar and pestle. 2462g of grinded Fenugreek was found. The powders were kept in an air-tight container until use.

Diet formulation

Fenugreek supplemented Diet

0.25kg of fenugreek (*Trigonella foenum-graceum*) seeds supplement was combined with 9.75kg of standard animal feed making the total feed formulation compositions to be; fenugreek seed 2.5% and standard animal feed 97.5%. This supplemented feed was fed to the hyperlipidemic Wistar rats for 21 days.

Management of Experimental Animals

Thirty (20) Wistar rats were purchased from the animal house of Human Physiology Department, College of Medicine Ahmadu Bello University Zaria. The animals were kept in well aerated laboratory cages in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. The rats were given access to drinking water ad libitum during the experimental period.

Animal Grouping and Experimental Design Twenty (20) Wistar rats were divided in four groups n=5, hyperlipidaemia was induced using a single dose of P407 (1.5g/Kg per body weight) IP (Hetal and Dion, 2013) at the interval of three days.

Group 1: Normolipidaemic feed with normal diet.

Group 2: Serve as disease control were administered Poloxamer 407 1.5g/kg (IP) per body weight and normal feed.

Group 3: Hyperlipidaemic rat and received Cholestyramine 0.26g/kg per body weight and normal feed.

Group 4: Hyperlipidaemic rat and received P407 1.5g/kg per body weight and Fenugreek (2.5%) supplemented diet.



Induction of hyperlipidemia

The experimental animals were intraperitoneally injected with 1.5g/kg of poloxamer-407 (Hetal and Dion, 2013).

Collection and Preparation of Serum Samples for Biochemical Assays

Three weeks after the treatment period, all animals were fasted for 12 hours and sacrificed after using mild anesthesia with chloroform. Blood sample (about 4 ml) was collected by cardiac puncture.

Blood samples were collected in plain tubes and allowed to clot. The serum was separated by centrifugation at $2000 \times g$ for 10 minutes. The serum collected was used for laboratory assay of urea, creatinine, and electrolytes (sodium ion and potassium ion).

Quantitative Analysis of the Phytochemical Constituents

Preparation of Fat Free Sample: About 2 g of sample was weighed and defatted with 100 mL of diethyl ether using a Soxhlet apparatus for 2 hours.

Alkaloid determination using Harborne (1973) method. About 5g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was then filtered and the extract was then concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was then allowed to settle and the precipitate was then collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid which was dried and weighed (Harborne, 1998).

Flavonoid determination by the method of Boham and Kocipai-Abyazan (1994). About 10g of the supplement was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight (Boham and Kocipai-Abyazan, 1994).

Tannin determination by Van-Burden and Robinson (1981) method

About 500 mg of the sample was weighed into a 50 mL plastic bottle and 50 mL of distilled water was added and shaken for 1 hour on a mechanical shaker. This was then filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted out into a test tube and mixed with 2 mL of 0.1 M FeCl_3 in 0.1 N HCl and 0.008 M potassium ferrocyanide ($\text{C}_6\text{N}_6\text{FeK}_4$). The absorbance was measured at 120 nm within 10 minutes (Van-Burden and Robinson, 1981).

Determination of total phenols by spectrophotometric method;

The fat free sample was boiled with 50 mL of ether for the extraction of the phenolic component for 15 minutes. About 5 mL of the extract was pipetted into a 50 mL flask then 10 mL of distilled water was added. About 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added. The sample was made up to the mark and left to react for about 30 minutes for color development. The absorbance was measured at a wave length of 505 nm.

Saponin determination by the method of Obadoni and Ochuko (2001)

20g of the sample was weighed and put into a conical flask then 100 mL of 20% aqueous ethanol was added. The sample was heated over a hot water bath at 100°C for four hours with continuous stirring at about 55°C . The mixture was filtered and the residue was re-extracted with another 200 mL of 20% ethanol. The combined extract was reduced to 40 mL over water bath at about 90°C . The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added. The combined n-butanol extract was washed twice with 10 mL of 5% of aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight, the saponin content was calculated as percentage (Obadoni and Ochuko, 2001).

Oxalate determination by Day and Underwood (1996) method:

About 1g of the sample was weighed into 100ml conical flask and 75ml of 3N H_2SO_4 was added and stirred for 1hour. It was then filtered using Whatman No. 1 filter paper. From the filtrate,



25ml was taken and filter while hot (80°-90° C) against 0.1N KMnO₄ solution, faint pink colour persist for 30 seconds. The oxalate content was calculated.

$$\text{Oxalate (mg/100mg)} = T \times V \times D \times 100 \div M \times M (g)$$

Where T is the titre of KMnO₄ (ml)

V is the volume mass equivalent

D is the dilution factor

M is the molar equivalent of KMnO₄ redox reaction and

M is the mass of sample used

Estimation of Serum Electrolytes

Estimation of serum sodium ion

Principle: Serum sodium was estimated based on the modification of methods first describe by Maruna (1958) and Trinder (1951) in which sodium is precipitated as the triple salt, sodium magnesium uranyl acetate. With excess uranium then being reacted with ferrocyanide producing a chromophore whose absorbance varies inversely as concentration of sodium in the test specimen.

Reagent composition

Filtrate reagent; Uranyl Acetate 2.1 mM and magnesium acetate 20nM in ethyl alcohol,

Acid reagent; A diluted acetic acid

Sodium color reagent; potassium ferrocyanide, non-reactive stabilizers, and fillers

Sodium standard; sodium chloride solution (150 mEq/L).

Procedure

Filtrate preparation;

Test tubes were labeled as follows; blank, standard, control, test 1- test 30.

1. 1.0ml of filtrate reagent was pipette in to all test tubes,
2. 50ul of sample was added in to all test tubes and distilled water to the blank,
3. Tubes were shaken vigorously and mix continuously for about 3 minutes
4. Tubes were centrifuge at 1500g for 10 minutes and a supernatant fluid ready for test is gotten,
5. A second set of test tubes were labeled corresponding to the first labelled
6. 1.0ml of acid reagent was pipette to all test tubes
7. 50ul of supernatant was added to respective tubes and mix
8. 50ul of color reagent was added to all tubes and mix,
9. Spectrophotometer is zero with distilled water at 550 nm and finally absorbance of all tubes was read and recorded.

Conc. Sodium (mEq/L) = (Abs of blank-Abs of sample) / (Abs of blank-Abs of standard) * conc. of standard (mEq/L).

Estimation of serum potassium ion

Principle;

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension (Terri and Sesin, 1958). The turbidity of which is proportional to potassium concentration in range of 2-7 mEq/L.

Reagent contents;

Potassium reagent; sodium tetraphenylboron 2.1nm. Preservatives and thickening agents.

Potassium standard; equivalent to 4mEq/L.

Procedure

1. Test tubes were labeled as follows; blank, standard, control, test 1- test 30
2. 1.0ml of potassium reagent is pipette in to all tubes
3. 0.01ml (10ul) of sample was added to tube and mix allow to stand at room temperature for 3minute,

Then, spectrophotometer was zero with reagent blank at wave length of 500nm, and absorbance of all tubes are read and recorded.

Potassium conc. (mEq/L) = (Abs) of sample / ((Abs) of standard) * conc. of standard (mEq/L) (Vogel, 1960).

Statistical Analysis

Data collected were expressed as mean ± SEM. It was analyzed using one-way analysis of variance, ANOVA, and Tukey's post-hoc test was used to compare the level of significance between the control and treatment groups, using SPSS version 20.0. Values of P<0.05 were considered significant (Duncan et al., 1977).

Results

The results of quantitative phytochemical analysis of the supplements revealed the presence of flavonoids (4.1%), oxalate (2.0%), alkaloids (2.05%), tannins (0.1%), saponins (0.55%) and phenol (0.7%) among other bioactive compounds. While Table 2; shows the results of electrolytes, urea and creatinine levels/distribution in the experimental and control groups.

Table 2: Electrolytes Distribution in P- 407 Induced-Hyperlipidaemic Wistar Rats Treated with Fenugreek Seeds Supplements

Group	Urea (mmol/L)	Creatinine (μ mol/L)	Sodium (Na ⁺) (mEq/L)	Potassium (K ⁺) (mEq/L)
Group 1	2.30 \pm 0.22	42.54 \pm 3.02	133.75 \pm 2.67	2.88 \pm 0.38
Group 1	2.36 \pm 0.22	41.44 \pm 2.73	139.92 \pm 4.63	3.84 \pm 0.18
Group 3	1.58 \pm 0.24	25.36 \pm 1.98*	134.32 \pm 1.65	4.08 \pm 0.24
Group 4	2.46 \pm 0.25	33.82 \pm 1.86	131.72 \pm 2.05	2.82 \pm 0.21#

Significant values: <0.05= * in comparison to group 2; p<0.01=# in comparison to group 3.

Key:

- Group 1: represent Normal Control group treated with standard animal feed
 Group 2: represent disease control group administered P-407 (1.5g/kg) and treated with standard animal feed
 Group 3: represent standard drug group administered P-407 (1.5g/kg) + Cholestyramine (0.26 g/kg) fed with standard animal feed
 Group 4: supplement treated group, administered P-407 (1.5g/kg) + Fenugreek seed (2.5 %) supplemented diet

Discussion

Serum electrolytes have vital functions in regulation of water distribution between different bodily compartments and maintenance of pH within physiological range, other regulatory activity is along electrolytic reactions that occur within and across many biological membranes (Shah *et al.*, 2011). Altered electrolytes balance is secondary to poorly control hyperglycaemia, hyperlipidaemia and associated pathologies in many metabolic disorders including type-2 diabetes mellitus (T2DM). Elevated levels are associated to dehydration, while decreased levels are seen in condition such as salt losing nephritis, and diabetic acidosis. Diabetes ketoacidosis is a major cause of death in poorly manage diabetes mellitus (DM) (Schonfed and Lowellen, 1964). The association between plasma overload of nutrients such as hyperlipidaemia, hyperglycaemia and serum electrolytes is a complex one and is related to a number of factors and associated conditions (Liamis *et al.*, 2014; Shah *et al.*, 2011). The findings of the present study revealed a significant decrease in creatinine level in the supplement treated group compared to the disease untreated group, creatinine is of paramount important in clinically review of renal functions along with other electrolytes. Although there was no significant distortion of the electrolytes profile statistically, but biologically a slight shift on certain solutes have noticeable observed influence on pathophysiology in the diseased. This result indicates a fluid retention like activities inferred in the disease control group, this agree with the study of Garcia *et al.*, 2018. Hyperlipidaemia contribute to the advent of health

problems, most notable is generalized mild inflammation of adipose tissues and accumulation of fats by muscular tissues that leads to decreased fluid with higher osmolality content in the system. Recent studies have indicated that obesity imposes a hemodynamic overload to the kidney's substances handling capacity. Therefore, regulation of the creatinine level seen in the supplement treated group may be associated with a reversing manifestation of renal functional complications revealed in the supplement treated group. The supplement may have assisted this effects as a result of it richness in phytochemistry of medicinal value such as phenols, flavonoids saponins and other related phytochemicals (Rahmani *et al.*, 2014)) as observed in the preliminary studies which is supported by report of previous studies (Goyal, *et al.*, 2016; Iyer *et al.*, 2009).

Pre-clinical studies have shown a direct correlation between a high fat diet and the overweight/obesity among distinct animal models (Dalboge *et al.*, 2015; Lee *et al.*, 2014). A review involving 6,618 renal biopsies performed from 1986 to 2000, reported a ten-fold increase in the incidence of obesity-associated glomerulonephritis in 2000 when compared to those investigated in 1986 (Kambham *et al.*, 2001). Obesity imposes a haemodynamic burden to the kidneys, which is characterized by glomerular hyperfiltration and microalbuminuria. It was observed that laboratory animals with obesity induced by high fat diet presented an elevation of 30% on the glomerular filtration rate five weeks after the induction, along with an expansion of Bowman's

capsule and an augment of mesangial matrix after nine weeks (Henegar *et al.*, 2001).

In conclusion supplementation of fenugreek seeds to hyperlipidemic (P-407 induced) Wistar rats is associated with conservation of renal physiological capacity of creatinine handling.

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